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A STUDY OF THE VENTRICULAR SYSTOLE—SUBCLAVIAN INTERVAL, WITH A DISCUSSION OF THE PRESPHYGMIC PERIOD*

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INTRODUCTORY

The material on which this paper is based was obtained at the suggestion of Prof. Friedrich von Müller, in his wards at the *Allgemeine Krankenhaus, links der Isar*, in Munich. We intended originally to take as the subject of the paper, the presphygmic period, i. e., the time between the beginning of ventricular systole and the opening of the aortic valves. As our work advanced, however, we had reason to doubt the accuracy of the presphygmic period as determined by our method, or, indeed, by any present method (except possibly in the case of aneurysms), and we, therefore, decided to discuss primarily the V_s-S time, or the time passing between the beginning of ventricular systole, and the arrival of the pulse wave at a given point on the subclavian artery.

As we shall show, the presphygmic period obtained in the usual way varies directly as the V_s-S time, and, indeed, is nothing but the V_s-S time with a fairly constant fraction of time subtracted. The significance of the two is, therefore, the same, and, in spite of the doubtful accuracy of the presphygmic period, we have in every case determined it, and have tabulated it, together with the V_s-S time.

METHOD

The tracings were all made with the Jacquet sphygmograph, running at high speed, i. e., 1 cm. = 1/5 second. Unless otherwise stated, the patients were all in the recumbent position, and usually turned slightly toward the left side, in order to obtain the best possible cardiogram. Simultaneous tracings were made of the apex beat, subclavian or carotid, and radial pulses. When possible, we used the subclavian in preference to the carotid pulse. The site selected was either on the subclavian artery

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above the clavicle 10 to 11 cm. from the second costosternal junction, or on the carotid an average distance of 15 cm. from the same point.

When satisfactory tracings had been obtained, foot-points of required waves were marked off on the time-line with a pair of calipers and measured, and, to correct any error due to slight variations of speed, each 1/5 second division of the time line involved was also measured, and in this way exact times computed. In order to insure an accurate average, a number of measurements of each required interval was made in every

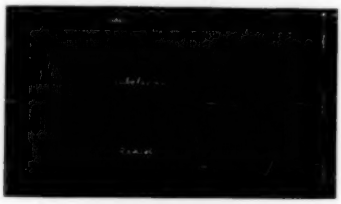

Fig. 1.—Case 15. Shows a cardiogram in which a jugular tracing (Tracing 2) helps to identify the correct foot-point.

instance, except in arrhythmias, where this is obviously impossible, and, unless the greatest variation was so slight as to fall within the limits of technical error, the tracings were discarded. The limit of technical error we called .01 second.

It is very hard in the majority of cases to obtain tracings in which the cardiogram and two pulses are all sufficiently definite and have sharp

Fig. 2.—Case 15. The "a" wave in the jugular tracing identifies the point V_s as the correct beginning of the upstroke in the cardiogram. That this point coincides with V_s in Tracing 1 is proved by the fact that the V_s — R times are the same in both tracings.

enough foot-points to use for purposes of measurement. The chief difficulty is perhaps encountered, as others[1] have pointed out, in getting a cardiogram in which there is a sharp foot-point, and about which there is no doubt as to where the true upstroke begins. This is particularly

1. Robinson, G. C., and Draper, G.: A study of the Presphygmic Period of the Heart. THE ARCHIVES INT. MED., 1910, v, 169.

hard and, in fact, usually impossible when the "auricular flip" is either very large, or absent. Occasionally simultaneous jugular tracings may settle the question (see Tracings 1 and 2), but as a rule, such cardiograms must be excluded.

The Ventricular Systole-Subclavian (V_s-S) and Ventricular Systole-Carotid (V_s-C) Intervals

The V_s-S time is obtained by making a simultaneous tracing of the apex beat and subclavian pulse, and calculating the time between the two foot-points in the manner described above. The V_s-C time is obtained in exactly the same way, except that the carotid pulse is used in place of the subclavian. The difference between the two is very small — the V_s-S time averaging .005 to .006 second less than the V_s-C time — which is of no practical importance, lying, as it does, within the limits of technical error. We found the V_s-S time the more satisfactory and have used it throughout, unless otherwise stated.

The V_s-S time represents the presphymic period plus the time taken by the pulse to travel from the aortic valves to the receiver on the subclavian artery. Variations in length may, therefore, represent changes in the presphymic period or pulse speed, or both. It has commonly been assumed that the presphygmic period was the main factor, and that the changes due to pulse speed could be ignored. Keyt[2] held that the V_s-C time and presphygmic period ran parallel, variations of one corresponding accurately to variations of the other. It seems, indeed, unlikely, as Keyt suggested, that the pulse in traveling 15 cm., which should take only about .021 second, could vary in speed sufficiently to cause any significant change in the V_s-C time.

The Relation of the Presphygmic Period to the V_s-S Time

Previous observers[3] have ranged the normal presphygmic period variously from .02 to .154 second. The methods most commonly used have been: (a) Measurement of the ascending limb of the cardiogram from

2. Keyt: Sphygmography and Cardiography. New York and London, 1887.
3. For references see Robinson and Draper. (Note 1.)

Observer	P. P. in Seconds	Observer	P. P. in Seconds
Jacquet and Metzner	.02 —.03	Rive	.073
Schmidt	.02 —.04	Hilbert	.06 —.12
Müller	.025—.07	Hoechaus	.07 —.10
Reck	.045—.06	Martius	.07 —.14
Tigerstedt	.051	Luciani	.08 —.10
Keyt	.06	Von Ziemssen and Maximovitch	.08 —.17
Hürthle	.06	Landois	.085
Einthoven and Geluk	.06	Edgren	.087—.093
Grunmach	.07	Erlanger	.12 —.18
Van Jürgen	.07	Robinson and Draper	07 —.085

the foot-point to notch P; (b) calculation from tracings of the pulse and the exposed heart; (c) measurement of the E-wave of aortic aneurysm; (d) obtaining the time between the foot-point of the cardiogram and the pulsation of aneurysm of the ascending aorta (probably the most accurate method); (e) calculation from tracings of the apex beat and the carotid or subclavian pulse, with or without correction for the time of pulse transmission. The last, with correction, which is the method we have used, is the one adopted by most recent observers, and is probably, with the exception of the aneurysm method, the most accurate.

Simultaneous tracings are made of the apex beat, subclavian or carotid, and radial pulses. The pulse speed is then obtained from the S-R (subclavian-radial) or C-R (carotid-radial) time, and from this the A-S time (pulse time from the aortic valves to the receiver on the subclavian) is computed. This subtracted from the V_v-S time gives the presphygmic period. The fallacy of this method lies in the assumption that the pulse speed is the same in the arm as in the thorax. Though Nicolai[4] thought that the pulse in the aorta and the carotid artery should be faster than in the arm, others[5] have shown quite conclusively that it is slower, and have suggested that in getting the presphygmic period too little had been subtracted from the V_v-C time. That not enough has been subtracted there can be little doubt, and we have some reason to believe, from work subsequently done by one of us (Swann), that a much greater delay may occur in the aorta than has heretofore been suspected, and that the presphygmic period, instead of being normally 0.07 to 0.085 second, as we have determined it to be by our present method, may be more nearly 0.055 to 0.07 second, which corresponds closely with v. Müller,[6] Reck,[7] Tigerstedt,[8] Keyt,[2] Hürthle[9] and Einthoven and Geluk.[10] Until some means is devised for recording the opening of the aortic valve, the present method will probably remain the most accurate at our disposal, and we have, therefore, in every case, calculated the presphygmic period in this way and tabulated it for comparison with the results of other observers.

4. Nicolai: Handbuch der Physiologie des Menschen, Brunswick, 1909, i, 787.

5. Keyt: (See Note 2) especially. Also Grunmach,[4] Robinson and Draper (Note 1), and Grunmach: Ueber die Pulsgeschwindigkeit bei Erkrankungen des Circulations-Apparates sowie bei Einwirkung toxischer Mittel. Virchows Arch. f. path. Anat., 1885, cii, 565.

6. v. Müller, F.: Einige Beobachtungen aus dem Percussionscurs. Berl. klin. Wchnschr., 1895, xxxvi, 783.

7. Reck: Graphische Untersuchungen über normale und pathologische Herzstossformen. Inaug. Diss. Bonn., 1890.

8. Tigerstedt: Die Puls-Kurve der Aorta beim Menschen. Skand. Arch. f. Physiol., 1908, xx, 249; Ergebn. der Physiol.-Weisbaden, 1902, i, Part 2, 253.

9. Hürthle: Beiträge zur Hämodynamik. Arch. f. d. ges. Physiol. (Pflüger's), 1891, xlix, 29.

10. Einthoven and Geluk: Die Registrirung der Herztöne. Arch. f. d. ges Physiol. (Pflüger's) 1894, lvii, 617.

The Subclavian-Radial (S-R) Time and Pulse Speed

After a careful investigation of the subject, Keyt[2] concluded that in the same individual the pulse speed in a given vessel varied but little with changes of pulse rate and other changes of the circulation, though varying greatly in different individuals, and somewhat in different vessels of the same individual. Our own observations[11] and those of others[12] have confirmed this.

For computing the pulse speed we have used, when possible, the subclavian and radial pulses, the distance between the two points used averaging 55 to 56 cm. The following tabulation shows the average pulse speed for all our cases with regular heart action:

Average speed — 7.09 metres per second, or 1 metre in .141 second.
Slowest speed — 4.52 metres per second, or 1 metre in .221 second.
Fastest speed — 10.31 metres per second, or 1 metre in .091 second.

Average S-R time —.082 second.
Longest S-R time —.126 second.
Shortest S-R time —.056 second.

Average A-S time (by calculation) —.015 second.*
Longest A-S time (by calculation) —.0219 second
Shortest A-S time (by calculation) —.0114 second.
Average A-C[16] time (by calculation) =.021 second.

* It is evident from the above figures that if we subtracted 0.15 second from the V.-S time in every case, we should have practically the same result as we obtain by the present method of determining the presphygmic period. For, if we substitute .015 second for the A-S time, as calculated in the two given cases, which represent the slowest and the fastest pulse speed obtained by us, we have errors of only .0036 second and .0069 second, respectively, which fall well within the limits of technical error.

Other observers have given the pulse speed as follows:

8.	metres per second.	Keyt.[2] Carotid-radial.
6.8	metres per second.	Erlanger.[13] Brachial and radial arteries.
6.15	metres per second.	Grunmach.[3] Heart to radial.
6.	metres per second.	Hürthle.[4]
9.42	metres per second.	Landois.[14] Arm arteries.
5.33	metres per second.	Jacquet and Metzner.[15] Innominate and carotid arteries.
8.33 — 20	metres per second.	Robinson and Draper.[1]

11. Two exceptions are Cases 17 and 21, in which the S-R time varied distinctly with changes of posture.

12. Garrod, A. H.: Some Points Connected with the Circulation of the Blood Arrived at from a Study of the Sphygmograph Trace. Proc. Roy. Soc. London, 1874, xxiii, 140; also Robinson and Draper, Note 1.

13. Erlanger: Cardiograms Obtained from a Case of Operative Defect in the Chest Wall. Bull. Johns Hopkins Hosp., 1905, xvi, 394.

14. Landois: Die Lehre vom Arterienpuls. Berlin, 1872, p. 304.

15. Jacquet and Metzner: Cardiographische Untersuchungen an einem Falle von Fissura Sterni. Deutsch. Arch. f. klin. Med., 1901, lxx, 57.

16. Keyt (Note 2) made the average A-C time .026 second, seven inches from the second interspace. This would correspond to .022 second, 15 c.m. from this point, coinciding closely with our own figures.

A glance at the above figures and at Table 1 shows how widely the speed has varied in our different cases. It shows, moreover, a conspicuous lack of conformity to any rules. It bears no constant relation to the V_s-S time,[17] nor does it vary consistently with the strength of systole, with the pulse rate, nor with different valvular lesions. In fact, the only rule which does seem to exist is that high blood-pressure produces a high speed, and low blood-pressure a low one. Thus Case 22, with the slowest speed, has a blood-pressure of 95/75, while Case 17, with the greatest speed, has a pressure of 190/170. This occurs in the majority of cases, although there are occasional exceptions.

RESULTS OF PRESENT INVESTIGATION

The Normal V_s-S Time

As the V_s-S time varies somewhat in different individuals, it is difficult to set the normal limits very definitely. From results obtained in normal cases, and in cases in which, though not entirely normal there seemed to be no factors which should affect the V_s-S time, we have placed the normal time at from .085 to .10 second. Our normal presphygmic period would then be .015 second less, or .07 to .085 second, which corresponds exactly with the presphygmic period, as set by Robinson and Draper.[1]

The Relation of the V_s-S Time to Heart Muscle Efficiency

Certain abnormal conditions of the circulation produce marked and fairly constant variations in the length of the V_s-S time. Thus, inefficient heart muscle and certain valvular lesions tend to lengthen it, while arteriosclerosis and other valvular lesions tend to shorten it. It is markedly affected by changes in posture, and somewhat by extremes of pulse pressure. Two or more of these conditions may occur together, in which case the effect of one may be increased or offset by the effect of others.

The question of the V_s-S time in its relation to heart muscle efficiency is interesting, and may be of value in diagnosis and prognosis, as has been suggested by former observers.[1] As they have pointed out, however, arteriosclerosis and other disturbing factors which may coexist, often make it impossible to determine just how much influence any one factor exerts, the V_s-S time therefore, being of definite clinical value only in cases in which there is an absence of disturbing factors. There can be little doubt that the V_s-S time is lengthened whenever the heart muscle is unable to meet the demands put on it, and it is probable that an

17. Robinson and Draper (Note 1) found that in their cases it bore no relation to the presphygmic period.

abnormally long V_s-S time, if this time were always measured, would often be the first obtainable evidence of impending cardiac failure. Of interest in this connection is one of v. Müller's[6] aneurysm cases, in which the presphygmic period obtained from the cardiogram and aneurysmal pulsation was .04 to .05 second. Fourteen days later the aneurysm had increased in size and the cardiac force had diminished, and the pre-

Fig. 3.—Taken with the patient lying down. Shows a slight pulsus alternans and also alternate variations in the length of the V_s-S times.

sphygmic period had increased to .07 second. In our cases this relationship of V_s-S time to myocardial efficiency is perhaps best shown in the arrhythmias (Table 3), in which in each given case the V_s-S times vary according to the strength of the preceding systole and length of the preceding diastole, i. e., according to the degree of fatigue and recuperation of the heart muscle.

Fig. 4.—Case 21. Taken with the patient standing. Shows a marked pulsus alternans.

In the following case variations of the V_s-S time depend entirely on the strength of the preceding systoles.

CASE 21.—The patient was 54 years old; a painter by occupation. He gave a history of lues many years before, and of five attacks of lead colic. He had never had rheumatism. For two years he had been unable to do hard work because of palpitation, dyspnea and mild anginal pain.

The patient was not confined to bed, was not dyspneic or cyanotic, and was suffering no particular discomfort. Heart: The apex impulse was seen and felt in the fifth space, 11 c.m. to the left of the mid-line. Dulness corresponded. No enlargement upward or to the right was made out by percussion. The first sound was loud, the second sounds were clear. There were no accentuations and no murmurs.

When the man was lying down the pulse was 80, of good size and force, at times regular and at times very slightly "alternans" in character. When he

TABLE 1.—Cases with Regular Heart Action, Classified According to Diagnosis

Case No.	Age	Diagnosis	Blood-Pressure	Pulse-Rate	S-R Time	V_v-S or V_v-C Time	P. P.	Speed per Meter
1	35	Normal heart	Normal	.....	.0950	.0890[c]	.0740	.155
2	20	Normal heart	135/105	70	.0970	.0948	.0787	.146
3	20	Normal heart	Normal	75	.0860	.0960	.0823	.139
4	26	Aortic regurgitation	165/125	75	.0781	.0679	.0556	.137
5	....	Aortic regurgitation	125/90	65	.1107	.0891	.0685	.190
6	37	Aortic regurgitation	170/115	63	.0764	.0847	.0687	.127
....	....	Same case standing	.....	75	.0759	.1100	.0962	.126
7	27	Aortic regurg. with great hypertrophy	.....	.....	.1000	.0870	.0690	.185
....	....	Same case standing	.....	.....	.0980	.1010	.0830	.181
8	46	Aortic regurgitation and stenosis	170/130	64	.0990	.0927	.0761	.165
9	19	Aortic regurgitation and mitral stenosis with relative regurgitation	120/90	80	.0860	.0870	.0730	.148
10	36	Aortic regurg. and mitral stenosis...	.....	70	.0954	.1006	.0836	.153
11	36	Aortic stenosis.....	105/70	45	.0674	.0920	.0813	.110
12	29	Mitral regurgitation	135/105	96	.0810	.0917	.0762	.147
13	40	Mitral regurgitation.	125/90	72	.1052	.1074	.0872	.166
14	37	Mitral regurgitation.	90/60	60	.0974	.1115	.0941	.157
15	17	Mitral regurgitation.	118/70	80	.0685	.1109	.1004	.118
16	26	Mitral regurgitation and stenosis	120/90	100	.0708	.1113	.0988	.131
17	42	"Beer heart," formerly acute dilatation; now compensated	190/170	60	.0664	.1175	.1061	.114
		Same case standing	170/155	60	.0563	.1547	.1450	.097
18	32	Chr. diffuse neph.; cardiac hypertrophy	.....	100	.0568	.0849	.0759	.088
19	27	Probable patent ductus arteriosus....	.....	68	.0978	.0895	.0728	.153
20	60	Coronary sclerosis with ang. pectoris	120/75	85	.0732	.0826	.0714	.112
21	54	Myocarditis and arteriosclerosis; chronic plumbism; pulsus alternans...	155/120	80	.0826	.0828	.0676	.137
		Same case standing	135/100	120	.0590	.1381	.1283	.098
22	17	Convalescent from acute tonsillitis.....	95/75	68	.1260	.0735	.0504	.221

S-R Time = time taken by pulse wave to travel from subclavian to radial artery.

V_v-S Time = Ventricular systole — subclavian interval.

V_v-C Time = Ventricular systole — carotid interval.

P. P. = Presphygmic period.

stood up the pulse rose to 120, became markedly "alternans" and thus remained as long as the patient was standing. The radial artery was much thickened. For blood-pressures see Table 2.

The diagnosis was chronic plumbism, arteriosclerosis and myocarditis.

Tracings taken with the patient lying down (Fig. 3), showed a slightly alternating radial pulse, and a V_s-S time which also alternated in length; being .0074 second shorter with the strong beats than with the weak ones; a difference which though very slight, was constant.

Fig. 5.—Case 21. Taken with the patient standing. Shows a marked pulsus alternans and great alternate variations in the length of the V_s-S times.

Tracings taken with the patient standing (Figs. 4 and 5), showed a striking increase in these differences, for not only was the character of the radial pulse accentuated, but the V_s-S time had an average alternation of .042 second, a variation more than five times as great as that obtained with the patient lying down. (Table 2.)

TABLE 2.—Case 21. Myocarditis and Arteriosclerosis; Chronic Plumbism; Pulsus Alternans

Position	Blood Pressure		Pulse Rate		Average V_s-S Time	Average P. P.	Difference
	Arm	Leg					
Lying	155/120	210/140	80	with weak beats	.0843	.0706	
Lying	155/120	210/140		with strong beats	.0769	.0632	.0074
Standing	135/100	250/ ?		with weak beats	.1591	.1493	
Standing	135/100	250/ ?	120	with strong beats	.1171	.1073	.0420

Table 2 shows a case with weakened contractility, in which the diastoles are equal, and the variations in the V_s-S time depend entirely on the strength of the preceding systole. It is an interesting fact that the alternate variations of the V_s-S time are greatly increased when the patient is standing. The mean lengthening which is present on standing, occurs to some degree in all cases and will be fully described later.

In this case, therefore, where there is every reason to suspect a heart muscle with impaired contractility, we have a V_s-S time which alternates in length — the length evidently depending entirely on the amount of

force expended in the preceding systole. The diastoles in this case play no rôle, for, although at first sight they appear to alternate in length, they prove on actual measurement to be equal, the apparent variations being due to differences in the V_a-S time.[18]

TABLE 3.—Showing Variations in the V_a-S Time

Case No.	Age	Diagnosis	Blood-Pressure	Pulse-Rate	Preceding Systole	Preceding Diastole	V_a-S or V_a-C Time	P. P.	S-R Time
23	25	Mitral regurgitation and stenosis, auricular fibrillation	125/90	90	S.	.272	.140	.128	.070
					W.	.184	.130	.116	.081
					W.	.187	.126	.113	.076
					S.	.322	.120	.108	.069
					X —				
					W.	.516	.096	.084	.072
					W.	.615	.096	.084	.068
					W.	.517	.096	.082	.080
					S.	.623	.090	.077	.078
					W.	.588	.086	.072	.084
24	60	Aortic regurgitation, arteriosclerosis, cardiac dilatation, auricular fibrillation	160/85	100	S.	.100	.200	.190	.053
					S.	.125	.171	.159	.060
					W.	.131	.159	.149	.048
					S.	.171	.158	.149	.048
					W.	.148	.141	.132	.046
					W.	.314	.131	.122	.051
					X —				
					W.	.526	.114	.104	.051
					W.	.761	.110	.099	.060
					W.	.432	.107	.098	.048
					W.	.553	.106	.097	.048
					W.	.678	.102	.092	.051
25	46	Myocarditis, auricular fibrillation	126/105	70	S.—	.381	.140	.128	.063
					S.—	.399	.132	.120	.066
					S.—	.473	.127	.116	.060
					S.—	.534	.127	.116	.064
					W.	.336	.126	.114	0.68
					X —				
					S.—	.605	.105	.095	.056
					W.	.779	.092	.081	.066
26	47	Mitral regurgitation and possible mitral stenosis, auricular fibrillation		114	S.+	.310	.153	.140	.071
					W.+	.234	.138	.126	.071
					S.—	.402	.129	.118	.071
					W.	.322	.118	.108	.065
					W.	.314	.118	.106	.075
					X —				
					W.	.610	.105	.090	.075
					S.—	.736	.089	.077	.075

18. Diastoles have been determined by measuring from the lowest part of the dicrotic notch of the subclavian or carotid tracings to the following foot-point, and deducting the presphygmic period. The diastoles in this case average .218 second before the weak beats, and .210 second before the strong ones, this slight discrepancy being probably due to technical error. Without correction they alternate by a difference of .05 second.

TABLE 3—CONTINUED

Case No.	Age	Diagnosis	Blood-Pressure	Pulse-Rate	Preceding Systole	Preceding Diastole	V_s-S or V_v-C Time	P. P.	S-R Time
27	72	Myocarditis and arteriosclerosis, auricular fibrillation.	210/160	90	S.	.220	.110	.100	.059
					S.	.220	.108	.098	.049
					W.+	.287	.101	.093	.048
					S.	.299	.100	.091	.051
					S.	.311	.098	.086	.053
					X —				
					W.	.330	.089	.080	.051
					S.	.784	.086	.076	.057
					S.	.435	.084	.075	.053
					W.+	.467	.082	.073	.050
					S.	.528	.082	.072	.059
					W.	.349	.080	.071	.053
					W.+	.360	.078	.070	.047
28	69	Myocarditis and arteriosclerosis, auricular fibrillation	155/125	70	S.	.230	.096	.080	.090
					S.	.274	.082	.066	.090
					S.	.376	.079	.064	.080
					W.	.522	.072	.058	.082
					W.+	.425	.069	.055	.082
					W.	.505	.071	.055	.096
					W.	.566	.068	.054	.080
					S.	.552	.065	.048	.098
29	67	Mitral regurgitation, arteriosclerosis, auricular fibrillation	145/120	98	W.+	.194	.095	.086	.056
					S.—	.325	.084	.075	.055
					W.	.200	.080	.070	.059
					S.	.331	.078	.069	.055
					S.—	.373	.074	.067	.045
					S.	.273	.074	.066	.049
					S.—	.457	.071	.062	.051

Table 3 shows marked variations of the V_s-S time, depending on the length of the preceding diastole and the strength of the preceding systole.

In Cases 23 to 26, point "x" denotes the shortest diastole which seems to be required for the heart to regain its full power. Below this point, even with longer diastoles, the V_s-S time varies but little.

The mean V_s-S time is long, except in cases in which arteriosclerosis is a marked factor, as in Cases 27, 28 and 29.

The variation of the S-R time is largely due to technical error, which, on account of the extreme difficulty of getting sharp radial foot-points, is bound to be somewhat greater than in cases with regular heart action.

ARRHYTHMIAS

After much difficulty we obtained satisfactory tracings in seven cases of absolute arrhythmia. In each case the V_s-S time varied widely in different curves, evidently depending on the degree to which the heart exhausted itself in systole, and in turn recuperated in diastole. Our

widest variation in any one case was from .102 to .200 seconds — the V_s-S time thus nearly doubling itself (Case 24). We found, as pointed out in regard to the presphygmic period by Robinson and Draper,[1] that the length of the V_s-S time seemed to depend on:

(*a*) The length of the preceding diastole; i. e., the shorter the diastole, the longer the V_s-S time.

(*b*) The strength of the preceding systole or systoles; i. e., the more force expended, the longer would be the following V_s-S time. This is shown in Table 3.

In Table 3 it is seen that as diastole grows shorter the V_s-S time rapidly lengthens. The opposite, however, seems to hold good only to a certain point. Some of our tracings strongly suggest that after a diastole of a certain length the heart has recovered its full ability, and that what slight shortening of the V_s-S time occurs after diastoles of still greater length may be due alone to further lowering of end diastolic pressure.

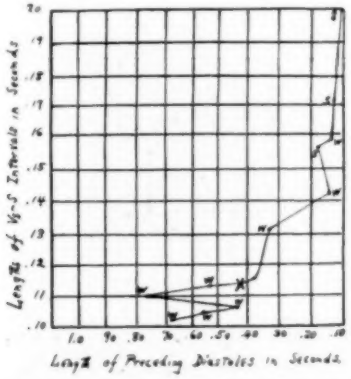

Fig. 6.—Case 24, showing relation of V_s-S time to length of preceding diastole and force of preceding systole. "S" denotes strong, "W" denotes weak preceding systole. Point "X" marks the shortest diastole which is apparently necessary for the heart to regain its full power. It is seen that to the left of this point the V_s-S time is fairly constant, whereas, to the right it varies widely, according to the length of diastole and strength of preceding systole.

For example, in Case 23 in Table 3, this point is seen to lie at "x"; i. e., somewhere between a diastole of .322 and .516 second. Above this line, following diastoles of less than .322 second, the V_s-S time varies widely as diastole and force of systole change; whereas, below the line, with diastoles above .516 second, they vary but little. Again, in Case 27, this point seems to lie between diastoles of .311 and .330 second. In Case 24 it lies between .314 and .432 second.

In all three of these cases, therefore, the time required for complete recuperation appears to lie between .311 and .526 second, and, if every heart were the same, we might place it between .322 and .330 second.

There can be little doubt, however, that different hearts vary considerably in this respect, as in the other cases we were unable to locate this point as definitely.

Fig. 7.—Case 23. Showing the influence of the preceding diastole on the length of the V_s-C time.

Curve 2. V_s-C=.126 sec. Diastole=.311 sec. C-R=.068 sec.
Curve 1. V_s-C=.120 sec. Diastole=.346 sec. C-R=.066 sec.
Curve 3. V_s-C=.098 sec. Diastole=.776 sec. C-R=.068 sec.

Fig. 8. Case 23. Venous tracing, showing absence of "A" wave.

Fig. 9.—Case 24, showing the influence of the preceding diastole on the length of the V_s-S time.

Curve 2. V_s-S=.170 sec. Diastole=.125 sec. S-R=.060 sec.
Curve 1. V_s-S=.131 sec. Diastole=.314 sec. S-R=.051 sec.
Curve 3. V_s-S=.110 sec. Diastole=.761 sec. S-R=.060 sec.

Influence of Posture on the V_s-S Time

To ascertain the effect of posture on the V_s-S time, tracings were first taken with the patient lying down. The patient then stood with his arm extended, so that the forearm lay at the level of the apex impulse; he

remained standing for ten minutes, when tracings were again made. We succeeded in getting satisfactory tracings of five cases. In every case the V_5-S time was definitely lengthened when the patient was standing (Table 4).

TABLE 4.—Patients Lying and Standing

Case No.	Diagnosis	Blood-Pressure	Pulse-Rate	V_5-S or V_5-C Time	Difference	P. P.	Difference
17	"Beer heart," formerly acute dilatation, now compensated	190/170	60	.1175	.....	.1061	.....
	(Same case standing)	170/155	60	.1547	.0372	.1450	.0389
21	Myocarditis and arteriosclerosis; chronic plumbism; pulsus alternans	155/120	80	.0828	.....	.0676	.....
	(Same case standing)	135/100	120	.1381	.0553	.1283	.0607
7	Aortic regurgitation, with great hypertrophy	.....	.....	.0870	.....	.0690	.....
	(Same case standing)	.....	.....	.1010	.0140	.0830	.0140
9	Aortic regurgitation and mitral stenosis (with relative regurgitation)	120/90	80	.0870	.....	.0730	.....
	(Same case standing)	.....	80	.1020	.0150	.0880	.0150
6	Aortic regurgitation	170/115	60	.0847	.....	.0687	.....
	(Same case standing)	.....	75	.1100	.0253	.0962	.0275

The V_5-S time is in every case longer in the erect than in the recumbent position. The greatest change in the V_5-S time is seen in Cases 17 and 21, which had no valvular lesions, but whose hearts were clinically considered to be the least competent. The possible prognostic value of this marked change in the V_5-S time is obvious.

It is interesting to note that the two hearts — Cases 17 and 21 — which had no valvular lesions, but which we had reason to regard clinically as the least competent, show much the greatest lengthening of the V_5-S time (respectively, .0372 and .0553 second), whereas the hearts which, in spite of marked valvular lesions, seemed to have plenty of reserve force (Cases 6, 7 and 9), show a lengthening of only .014 to .025 second. Though we have reason to believe, from tracings obtained on normal cases standing, that a moderate lengthening of the V_5-S time constantly occurs, we were unfortunately not able to obtain figures which were accurate enough to record. The subject, however, should be further

investigated, for it is probable that a marked lengthening —such as is seen in Cases 17 and 21 — might prove to be of great prognostic value.

Whether this lengthening of the V_s-S time is due to a change in the presphygmic period, or to delay of the pulse in the aorta, we are not prepared to say definitely, though we think that the presphygmic period is probably the main factor. The S-R time, though in three cases unaffected by posture, was in the other two somewhat shorter when the

Fig. 10.—Case 17. Taken with the patient lying. Average V_s-S time .1175 second.

patients were standing. This means that, if there is any difference, the speed of the pulse in the arm is somewhat greater when standing than when lying down; and we would, therefore, not expect the speed in the aorta to be just the reverse. This point, however, is not at all certain.

As an explanation of the lengthening of the V_s-S time, we suggest that it is due to a sudden increase in the work imposed on the heart.

Fig. 11.—Case 17. Patient standing. Shows marked lengthening of V_s-S time. Average V_s-S time now .1547 second.

Inasmuch as the venous column of blood from the level of the heart down, is heavier in the erect posture than the arterial column, as the experiments of Mosso[19] have shown it to be, it is obvious that, to maintain the circulation, the heart has to work against greater resistance. This would tend to lengthen the presphygmic period and V_s-S time, and especially would this be the case when a heart with little reserve was not quite equal

19. Mosso: Application de la balance a l'étude de la circulation du sang chez l'homme. Arch Ital. d. biol., 1884, v. 130.

to the task imposed on it. In terms of blood-pressure, this is equally true. Table 1 shows some relation in the length of the V_s-S time to diastolic pressure, and a fairly constant relation to pulse pressure, a low pulse pressure being associated with a long V_s-S time and *vice versa*. The careful investigation of the effect of posture on blood-pressure by

Fig. 12.—Case 6. Aortic regurgitation. Good compensation. Patient lying. Average V_s-S time = .0847 second.

Fig. 13.—Case 6. Patient standing. Shows moderate lengthening of the V_s-S time, averaging .1100 second.

Fig. 14.—Case 14. Mitral regurgitation. Average V_s-S time = .1115 second. See also Tracing 11, Case 6, Aortic Regurgitation. Average V_s-S time = .0847 second.

Erlanger and Hooker[20] has shown that, when very accurate determinations are made, the diastolic pressure is usually higher, and the pulse pressure invariably lower in the erect than in the recumbent position. We should, therefore, rationally expect to find the V_s-S time lengthened, if affected at all, in the erect posture.

20. Erlanger and Hooker: Johns Hopkins Hosp. Rep., 1904, xii.

The Effect of Valvular Lesions on the V_s-S Time

Previous observers have differed as to the effect of various valve lesions on the V_s-S time and presphygmic period.[21] Our results go to show that, quite apart from other factors, aortic regurgitation tends to shorten and mitral lesions to lengthen the V_s-S time. Thus, in four cases of aortic regurgitation, the V_s-S times were either below or near the lower limits of normal, while in five mitral cases the times were all, with one exception, well above normal. The averages (see Table 5) show such striking differences that one is forced to believe that the V_s-S time is definitely affected by the nature of the lesion itself, as well as by the condition of the heart muscle.

TABLE 5.—Cases with Regular Heart Action; Classified According to Length of V_s-S Time

Diagnosis	No. of Cases	Average V_s-S Time	Average P. P.
"Beer heart"; formerly acute dilatation. Now compensated	1	.1175	.1061
Mitral regurgitation and stenosis	1	.1113	.0988
Mitral regurgitation	4	.1053	.0894
Aortic regurgitation and mitral stenosis	2	.0968	.0783
Normal heart	3	.0932	.0783
Aortic regurgitation and stenosis	1	.0927	.0760
Aortic stenosis	1	.0920	.0810
Probable patent ductus arteriosus	1	.0875	.0728
Chronic diffuse nephritis; cardiac hypertrophy	1	.0849	.0759
Myocarditis and arteriosclerosis; chronic plumbism; pulsus alternans	1	.0828	.0676
Coronary sclerosis and angina pectoris	1	.0826	.0714
Aortic regurgitation	4	.0822	.0654
Convalescent from acute tonsilitis	1	.0735	.0504

Table 5 shows the effect of valvular lesions on the V_s-S time. It is seen that in mitral cases the average is above normal, while in cases of aortic regurgitation it is below normal. In the cases of aortic stenosis and congenital heart disease, the lesion seems to have had little or no effect.

21. Keyt (Note 2) held that the V_s-C time was shorter in aortic regurgitation than in any other condition, except in fever cases with rapidly acting hearts, and that theoretically it should be lengthened in mitral lesions, although he had not estimated it in any such cases. Hilbert (Beitrag zur Deutung der Herzstosscurve. Ztschr. f. klin. Med., 1891, xix, Supplement, 153), on the other hand thought the presphygmic period unaffected by the lesion in hearts with good compensation. Hochhaus (Beiträge zur Cardiographie. Arch. f. exper. Path. u. Pharmakol., 1893, xxxi, 405; Ueber frustrane Herzkontraktionen, München. med. Wchnschr., 1907, liv, 401), found that in all his cases of mitral regurgitation and mitral stenosis the presphygmic period fell within normal limits. (He gives as normal .07-.10 second.) Robinson and Draper' concluded that the length of the presphygmic period depended more on the ability of the heart to meet demands than on the lesion itself.

Minor Points Regarding the V_s-S Time

A marked degree of arteriosclerosis tends to shorten the V_s-S time. This observation was made by Robinson and Draper[1] in regard to the presphygmic period. Our cases have confirmed this, as is shown by Case 21 in Table 1, and by Cases 27, 28 and 29 in Table 3.

We found that the V_s-S time bore no constant relation to pulse rate.[22]

Though the effect of blood-pressure is not as constant as we might expect, our cases showed some relation of the V_s-S time to diastolic pressures; and this relationship was most definite in cases with a marked difference between the systolic and diastolic pressure (high pulse pressure) in which cases we nearly always obtained a short V_s-S time.

One might expect that a split "C" wave in the jugular tracing would be associated with a long V_s-S time and presphygmic period. Inasmuch as the first part of the split wave — the "VK" wave of the Germans —

Fig. 15.—Case 11. Aortic stenosis. Average V_s-S time = .0920 second.

is probably due to impact on the tricuspid flaps exerted in early ventricular systole, and is separated from the opening of the aortic valves by a portion of the presphygmic period, we should expect, in the case of a long presphygmic period, to have a well-marked "VK-C" interval. In none of our cases, however, which showed a split "C" wave in the jugular tracing, was there a V_s-S time or presphygmic period above normal. (See Table 6.)

CONCLUSIONS

1. The normal V_s-S time is from .085 to .10 second. It varies widely in certain abnormal conditions of the heart and circulation.

2. The presphygmic period, as determined by calculation from simultaneous tracings of the apex beat, subclavian and radial pulses, is about .015 second shorter than the V_s-S time, and runs parallel to it.

22. Though Carrol (Note 12) thought that the presphygmic period was short in cases with a rapid pulse rate and *vice versa*, our cases failed to show any constant relationship between the pulse rate and the V_s-S time. Our observations agree with those previously made in regard to the presphygmic period by Hürthle (Note 9) and by Robinson and Draper (Note 1).

variations in one fairly accurately representing variations in the other. We doubt the accuracy of the presphygmic period as obtained by this method and hope to settle this point more definitely in future work.

3. In cases of auricular fibrillation the variations in the V_s-S time depend on: (a) the length of the preceding diastole, and (b) the strength of the preceding systole or systoles; being longest after a short diastole, and a strong systole, and *vice versa*. After diastoles of a certain length, however, the length of the V_s-S time varies little or not at all. From figures obtained in three cases, the length of diastole necessary for complete rest apparently lay between .322 and .330 second.

4. In cases of broken compensation, the mean V_s-S time was invariably lengthened, except when a marked degree of arteriosclerosis was present. It is probable that, when no disturbing factors coexist, the length of the V_s-S time may be of value in estimating the capability of the heart muscle.

TABLE 6.—SHOWS THAT A SPLIT "C" WAVE IN THE JUGULAR TRACINGS WAS NOT ASSOCIATED WITH A LENGTHENED V_s-S TIME

Case 1.	Average VK-C interval = .032	V_s-S = .0890	P. P. = .0740
	Average A -C interval = .205		
Case 8.	Average VK-C interval = .012	V_s-S = .0927	P. P. = .0761
Case 12.	Average VK-C interval = .029	V_s-S = .0917	P. P. = .0810
	Average A -C interval = .181		
Case 19.	Average VK-C interval = .053	V_s-S = .0895	P. P. = .0728
	Average A -C interval = .277		

5. In every one of five cases on which tracings were taken with the patients lying and standing, the V_s-S time was definitely longer in the erect posture. As by far the greatest lengthening occurred in the two cases which were clinically regarded as having the least competent hearts, it is probable that the degree of lengthening may prove to be of value in prognosis.

6. Aortic regurgitation shortens, and mitral valve lesions lengthen, the V_s-S time. Combined lesions, having opposite effects, tend to offset one another.

7. A marked degree of arteriosclerosis shortens the V_s-S time.

8. The relation of the V_s-S time to blood-pressure is not constant. It tends, however, to become shortened when the pulse pressure is high, especially if at the same time, the diastolic pressure is low. The opposite also holds good.

9. Split "C" waves in the jugular tracings were not associated with abnormally long V_s-S times.

10. The speed of the pulse wave in the arm varies with extremes of blood-pressures, being faster in cases with high, and slower in cases with low, blood-pressure. This relationship, however, is not constant. No

other factors seem to have any definite effect on the pulse speed. Although it varies widely in different individuals, it is nearly always constant in a given vessel of the same individual.

In conclusion we wish to express our thanks to Professor Friedrich v. Müller for the splendid material afforded us, and to Dr. Ernst Edens for his aid in the interpretations of tracings and for many helpful suggestions. We also wish to thank the assistants of the Second Medical Clinic for many courtesies extended to us in the wards.

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THE RELATION OF THE HYPOPHYSIS TO GROWTH AND THE EFFECT OF FEEDING ANTERIOR AND POSTERIOR LOBE *

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As observed by the clinician, there are two clinical entities, both disturbances of growth, ascribed to disturbed functioning of the hypophysis. One is acromegaly, characterized by enlargement of certain bones; the other, delayed development with adiposity and genital atrophy. When the former occurs in early life, gigantism results; when it first appears after maturity, enlargement of only certain portions of the bony skeleton is observed. In the other type, when the disturbance appears in early life, there is delayed skeletal development, with adiposity and failure of sexual evolution; when it appears after maturity, adiposity and sexual atrophy.

Pierre Marie, in 1886, first called attention to the relation between acromegaly and the hypophysis, although Carl von Langer in 1872, in an anatomical study of giants, referred to a certain type with enlarged sella turcica. The observations of Marie have been confirmed, until at present it is generally conceded that acromegaly is due to a disturbance of the hypophysis. According to Sternberg, 40 per cent. of the pathologic giants have an enlargement of this organ. Regarding the exact nature of the disturbance in the hypophysis in acromegaly, there is still considerable difference of opinion. The weight of evidence, however, favors the view that it is due to hypersecretion of the anterior lobe. The pathologic condition most frequently associated with acromegaly is an adenomatous development of the anterior lobe with an increase in the specific secretory cells. In some instances in which enlargement of the anterior lobe is lacking, increase in the specific secretory cells may still be demonstrated, and in addition hyperplasia of the pharyngeal hypophysis should be considered in these cases. There are reported in the literature, malignant tumors of the hypophysis with acromegaly. Lewis, who has reviewed these cases, believes that in the majority, and possibly in all, of these the tumor was an adenoma. Although it is too early to state positively that acromegaly is due to hypersecretion from the anterior lobe, it must be admitted that the weight of evidence supports this view.

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* From the Department of Experimental Therapeutics, University of Chicago, S. A. Matthews, director.
* Presented at the Eighth International Congress of Applied Chemistry.

The condition of underdevelopment, adiposity and genital atrophy, first described by Fröhlich and referred to as the Fröhlich syndrome, is usually associated with disturbances at the base of the brain, more especially with tumors. Whether in all of these cases the hypophysis is involved, either directly or indirectly by either increased intracranial or intracerebral pressure, has not been determined. On account of its position, the hypophysis is especially liable to be influenced when there is an internal hydrocephalus, and it is quite possible that any cerebral disturbance causing increased pressure in the ventricle may compress and disturb the hypophysis. There is one case on record in which a bullet wound of the hypophysis was followed by adiposity (Madelung). Adiposity is associated with acromegaly, according to Creutzfeldt, in 1.6 per cent. of cases. In five of the recorded cases of adiposis dolorosa, with autopsy, the hypophysis has been abnormal (Lyon). Cases are also on record in which removal of the hypophyseal tumor was followed by disappearance of the adiposity (Von Eiselberg).

Assuming that adiposity may arise from disturbances of the hypophysis, it is still to be determined what portion of the glands is responsible for these changes. Fischer believes it is due to hyposecretion of the posterior lobe. In addition to certain theoretical considerations, he presents some autopsy findings in which as the result of pressure, the posterior lobe was flattened and had undergone brown atrophy. It can readily be conceived, however, that any pressure involving the posterior lobe must also compress the anterior portion. In all of Fischer's evidence there is little that is actually convincing. A more tenable view is that hyposecretion of the anterior lobe is responsible for the adiposity. Zollner, in a case of this type, found a carcinoma of the anterior lobe. In the five cases of adiposis dolorosa, referred to, with hypophyseal involvement, two showed round-cell infiltration of the anterior lobe (Price), one a glioma evidently of the posterior lobe (Burr), one marked increase in size of the anterior lobe, due to a connective tissue hyperplasia and an alveolar sarcoma (Guillain), and one (Dercum's case) a carcinoma involving both anterior and posterior lobes. Although few deductions may be drawn from the above evidence, on the whole it favors disturbance of the anterior lobe. Most convincing, however, is the experimental evidence. In dogs, removal of the posterior lobe is not followed by any serious consequence, the animal recovers and later fails to show any anomalies of growth (Paulesco, Cushing, Ascola). Where a considerable portion of the anterior lobe of a young dog is removed and the animal recovers from the effect of the operation, he later develops the Fröhlich syndrome of delayed development, adiposity and failure of sexual development (Aschner, Cushing, Casselli, Ascola). This evidence is most suggestive and points strongly toward hyposecretion of the anterior lobe

as the cause of the adiposity and sexual atrophy. The question may properly be raised whether the adiposity is due directly to the lessened secretion of the anterior lobe or is secondary to the genital atrophy, for Tandler and Grosz have shown that castration in dogs leads to increased deposits of fat, and this phenomenon is observed in women after removal of the ovaries. It might be argued that it would be exceedingly difficult on the basis of hyposecretion of the anterior lobe to explain the occasional presence of adiposity in acromegaly. Such adiposity is very infrequent, occurring, according to Creutzfeldt, in only 1.7 per cent. of cases, while hypoplasia and genital atrophy were noted in 36.4 per cent. of the cases. The greater frequency of the sexual disturbance would lead us to suspect that it and the adiposity were due to separate factors. Here, again, the possibility of the adiposity being secondary to the genital atrophy must be considered, its inconstant associations being due to the varying degrees of sexual hypoplasia in the various cases. To those who believe that the adiposity is due to the hyposecretion of the posterior lobe, its presence in acromegaly is explained by pressure of the enlarged anterior lobe on the posterior lobe.

Summarizing, it may be said that acromegaly is probably due to increased activity of the anterior lobe. Adiposity, if due directly to disturbances of the hypophysis, is most probably due to hyposecretion of the anterior lobe.

METABOLISM IN ACROMEGALY

The metabolism in acromegaly has now been studied in a dozen or more cases. Retention of nitrogen is very frequent, often reaching considerable amounts. In some of these cases calcium and phosphorus metabolism is unchanged; in others, retention of both calcium and phosphorus have been reported. Seven cases from five different observers all show a retention of nitrogen, five of phosphorus and five of calcium. Oberndörffer has recently reported two cases and reviewed the literature on this subject. He was unable to detect any variation from the normal in his two cases, and questions whether the results of others are really conclusive, on account of the great variations in elimination of normal individuals. Before the work on this phase of the subject can be accepted, more extensive studies should be carried out.

METABOLISM IN ANIMALS AFTER THE ADMINISTRATION OF HYPOPHYSIS

Thompson and Johnson fed dogs on the entire dried gland from the horse, calf and sheep, and found that they lost in weight and excreted an excessive amount of nitrogen and phosphorus (ca. and mg. not determined). They also reported more marked results when glands of young animals were used. Malcomb gave dogs 2 to 3 gm. daily of dried

anterior lobe, for a period of five days, a total of 15 gm. The animals showed slight retention of nitrogen and slightly increased output of P_2O_5 and Ca. After feeding posterior lobe (10 gm.) there occurred a slightly increased output of P_2O_5 and marked increased output of Ca. When he gave fresh entire glands, 25 gm. daily, there was scarcely any change in the Ca or Mg. output. Franchini injected rabbits, intravenously, daily with an amount of extract equivalent to one entire hypophysis. This was followed by greater elimination of Ca, Mg. and P_2O_5 in both urine and feces, the loss in P_2O_5 being less marked than of Ca or Mg. The animals lost in weight and finally died. The x-ray failed to show any change in the bony skeleton. Some of the animals tolerated the injection well; others showed marked dyspnea, vomiting and diarrhea. Franchini's conclusions that hyperpituitarism leads to loss in weight and failure of development is scarcely justified on account of the severe reaction following his intravenous injections. Oswald gave dogs 2 to 3 gm. daily of dried hypophyseal extract obtained from Merck (portion of gland not specified) and was unable to detect any change in nitrogen or P_2O_5 elimination. Benedict and Homans, working with hypophysectomized dogs and using carbon dioxid production as an index of total metabolism, found it markedly reduced.

The results of these findings vary so much that they throw little light on the disturbance of metabolism following administration of dried hypophysis and may be regarded as furnishing no definite evidence.

FEEDING EXPERIMENTS

Comparatively few satisfactory feeding experiments have been reported. In some, no attempt has been made to feed the two lobes separately. Others have administered the extract subcutaneously or intravenously (Cerletti, Franchini, Delille, Caselli). When given in this way it frequently gives rise to marked constitutional disturbances, such as vomiting and diarrhea, and, finally, intestinal ulceration, that it is impossible to draw any conclusion regarding the actual effect of the hypophyseal extract. After this method of administration, Cerletti and Franchini report loss in weight and delayed bone development. The entire hypophysis was used and the animals treated for a few days only. Caselli injected young dogs and rabbits with glycerin extracts and did not notice any effect on growth. Delille injected extracts of the entire hypophysis into four rabbits for a period of fourteen months and reported increased deposits of fat.

Only two references to feeding experiments have been obtained in which the animals received preparations of the hypophysis by mouth for a considerable period of time.

Sandri fed rats on hypophysis exclusively for a period of two months, the controls receiving an exclusive meat diet. While this is an unsuitable

diet, Sandri reports that the animals thrived. He found that those fed on the anterior lobe showed greater gain in weight than the controls. When we consult the actual figures, we find that these differences are so slight that they can scarcely be considered as significant. The controls during the two months gained, on an average, 10 gm.; those fed on the posterior lobe, 7 gm.; those fed on the anterior lobe, 12 gm. Variation of this degree may occur in any group of feeding experiments continued over a period of three months.

Schaefer has conducted the most satisfactory feeding experiments, using, however, only the anterior lobe. Four young rats were fed small amounts of the dried anterior lobe, mixed with bread and milk. The controls received powdered testicle or ovary, with bread and milk. The amount consumed by each group of animals was accurately determined. The feeding experiment was continued for about three months. At the beginning, the average weight of the group fed on hypophysis was 44.25 gm., and that of the controls exactly the same. At the end of the feeding, the average weight of those fed on hypophysis was 160 gm., and of the controls, 131 gm. During the first six weeks of the feeding, there was little difference between the two groups; during the last six weeks those fed on hypophysis made the more rapid gain. These results would appear to be conclusive; they are not, however, sufficiently numerous to eliminate error.

AUTHORS' EXPERIMENTS

In undertaking this investigation, it was decided to carry through several series of animals with controls. Young white rats were selected. Each rat was placed in an individual cage. For food, ground cracker was chosen. This was pressed into tablets, each of the same weight. It was first determined how much of this food each rat would consume daily. Although there were some individual differences, it was possible to determine with reasonable accuracy the daily ration. Having determined this point, cracker tablets of the requisite weight were made, and to each was added a weighed amount of the hypophysis, or in case of the control, meat, and each animal received the same amount daily. Occasionally, for a few days, a rat might not eat his whole tablet; a note was made of this fact. However, the ration was so arranged that, with rare exceptions, it was consumed daily. No doubt some of the animals would have eaten more. The fact that they gained in weight and appeared on the whole healthy would, however, indicate that they were properly fed. By this method, each rat received and consumed the same amount of food daily, containing the same amount of the substance to be tested. The animals were weighed each week.

The ox hypophyses were obtained perfectly fresh from the Union Stock Yards. The anterior and posterior lobes were separated, chopped

up fine and dried in a blower at a temperature of approximately 100 F. The dried glands were then powdered and a weighed amount added to the powdered cracker and pressed into a tablet. Three series were fed in this way for about three months each; at the end of the time the rats were killed and radiographs taken to detect any changes in the bony skeleton. The first series consisted of nine rats. Three received daily 2 gm. of dried anterior lobe, three the same amount of posterior lobe and three controls the same amount of meat. The feeding was continued for seventy-nine days. The second series consisted of eight rats. Four received 4 gm. anterior lobe and the other four as controls received the same amount of dried meat. This group was fed for ninety days. The third series of nine young rats was divided into three groups. One group received 3 gm. daily of beef, another the same amount of posterior lobe, and the other the same amount of dried thymus. This series was kept under observation for sixty-seven days. By repeating the experiment in this manner, it was thought that some sources of error might be eliminated.

TABLE 1.—Results of Feeding Experiments in Rats

Number of Animals	Food Daily, Gm.	Average Weight at Beginning, Gm.	Average Weight at Termination, Gm.	Change in Weight, Gm.	Period of Feeding, Days.
	SERIES I				
3	Dried beef, .2	52.2	91.8	38.6	78
3	Dried anterior lobe, .2	54.2	92.3	37.9	78
3	Dried posterior lobe, .2	58.1	102.6	44.5	78
	SERIES II				
4	Dried beef, .4	58	95.2	37.2	90
4	Dried anterior lobe, 4	66.6	107.3	40.7	90
	SERIES III				
3	Dried beef, .3	131.6	144.3	12.6	67
3	Dried posterior lobe, .3	115.3	121.5	6.2	67
3	Dried thymus, .3	118.6	135.3	16.7	67

It is interesting to note that animals consuming the same amount of food daily and apparently enjoying equally good health, should show such marked variations of gain in weight. The minimum gain in weight of the controls in Series I was 30.6 gm., the maximum, 44.4 gm. In Series II the minimum gain in weight of the control rats was 32 gm., the maximum 38.5 gm. In Series III, wherein the rats were two-thirds grown, and, therefore, not so suited for the test, in both the controls and those fed on posterior lobe, one of the animals lost 5 gm.

As will be seen by the table in the first series, the controls and those fed on the anterior lobe showed practically the same gain in weight. Those fed on the posterior lobe, gained an average of 6 gm. each more than the controls. When we consider the individual animals, one of those fed on the posterior lobe gained less than one of the controls, the other two gained more than the controls and each animal fed on the posterior lobe gained more than those receiving the anterior lobe. In Series III, however, the animals receiving the posterior lobe gained less than the controls and much less than those animals receiving thymus. In Series I, animals receiving anterior lobe gained slightly less than the controls, while in Series II, they gained somewhat more than the controls. The radiographs of all these animals failed to reveal any variations in the bony skeleton.

Only one conclusion can be drawn from these feeding experiments, viz., that, at least in this series of tests, neither anterior nor posterior lobes had any effect on the weight or growth of the animal. The experiment was conducted in such a manner that serious causes of error were excluded. The amounts administered were sufficient to give results, as they would be equivalent to 230 gm. daily to the average man. On the other hand, the amounts were not sufficiently large to have a deleterious effect, as the animals so fed gained the same in weight as the controls. Doubling the dose of anterior lobe did not modify results. It must be admitted, however, that this does not prove that disturbed secretion of the hypophysis may not modify growth. In the feeding experiment, the digestive fluids may destroy the active substances responsible for these changes. Again, feeding preparations by mouth can scarcely be considered as analogous to the continuous secretion occurring in actual life.

SUMMARY

Summarizing the entire field of the rôle of the hypophysis in the growth of the individual in acromegaly in which there exists abnormal development of certain portions of the body, especially in their bony structures, there is apparently hypersecretion of the anterior lobe. In the Fröhlich syndrome of adiposity and failure of sexual development, it is thought by many that there is lessened function of the posterior lobe; experimental information, however, suggests lessened secretion of the anterior lobe, but evidence on this point is not especially convincing. Regarding studies in metabolism in patients with acromegaly, there is again nothing conclusive, and more work must be carried out on this subject before it can be accepted that lessened katabolism takes place as compared with the normal individual.

Turning to the results of partial removal of the hypophysis in animals, only one point having a direct bearing on this subject seems to

have been determined, viz., that partial removal of the anterior lobe, when performed on young animals, modifies growth and sexual development in such a manner as to resemble very closely the Fröhlich syndrome. Removal of the posterior lobe, apparently, has no effect on growth. This is a distinct contradiction to those who believe lessened function of the posterior lobe is responsible for the Fröhlich syndrome. Feeding experiments, on animals, fail to furnish any definite evidence that the administration of either the anterior or posterior lobe has any effect on growth.

We wish to express our thanks to Mr. E. M. Miller for his assistance in this investigation.

Since this work was completed, T. B. Aldrich under the title, Feeding Young Pups the Anterior Lobe of the Pituitary (Am. Jour. Physiol., 1912, xxx, 352), has reported negative results after feeding anterior lobe.

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A STUDY OF HYDROGEN ION CONCENTRATION OF THE URINE IN HEART DISEASE*

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BOSTON

The circulating fluids of the organism are practically neutral and nearly constant in reaction, and an animal, into whose body acid is being introduced, dies before his serum gives an appreciably acid reaction.[1] Nevertheless, the trend of metabolism is toward the production of acid greatly in excess of alkali; but under normal conditions the whole of this excess is separated from the blood in its passage through the kidney. One of us has shown the chemical mechanism which underlies

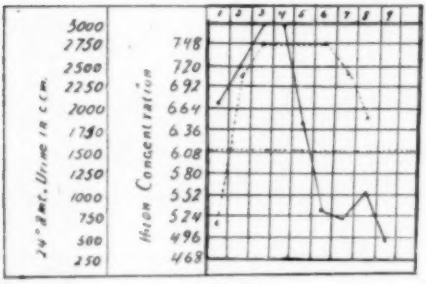

Chart 1.

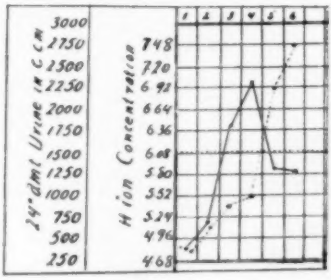

Chart 2.

Chart 1.—Case 1. Double hydrothorax; large ascites; marked general anasarca; arteriosclerosis; cardio-renal. Blood-pressure 160. Made a rapid and uneventful recovery.

Chart 2.—Case 2. Mitral disease. No hydrothorax, ascites or edema. Moist râles in lungs. Improved rapidly.

The solid line in this and other charts represents the twenty-four hour amount of urine in cubic centimeters. The broken line represents the hydrogen ion concentration in each twenty-four-hour specimen of urine expressed logarithmically. The horizontal broken line represents the mean of the hydrogen ion concentration of the twenty-four-hour specimens of urine from 150 normal persons.

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* Submitted for publication May 1, 1913.

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1. Walter, F.: Untersuchungen über die Wirkung der Sauren auf den thierischen Organismus, Arch f. exper. Path., 1877, vii, p. 148.

this neutrality preservation and has pointed out its far-reaching biological significance.[2]

Whether the acid can be removed as fast as it is produced in various abnormal conditions, and, the mechanism failing, what pathological states

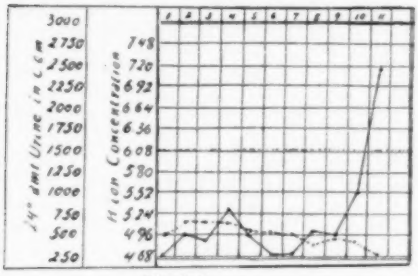

Chart 3.

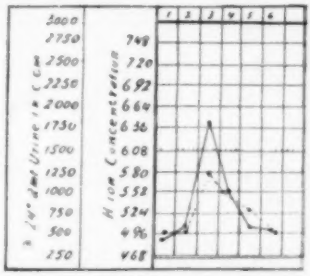

Chart 4.

Chart 3.—Case 3. Arteriosclerosis; weak dilated heart; marked edema of legs. Patient became subjectively comfortable but objective signs did not change.

Chart 4.—Case 4. Chronic nephritis. Blood-pressure 250; right hydrothorax; marked edema of legs; edema disappeared at time of diuresis. Otherwise no improvement.

may be thereby called into existence, are matters still open to investigation.

In order further to investigate this question, it seemed advisable in the first place to study the reaction of the urine when disturbance of the

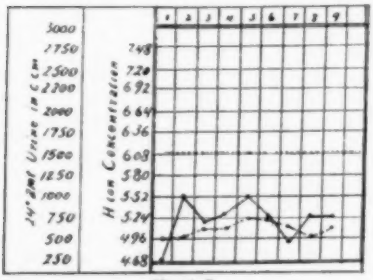

Chart 5.

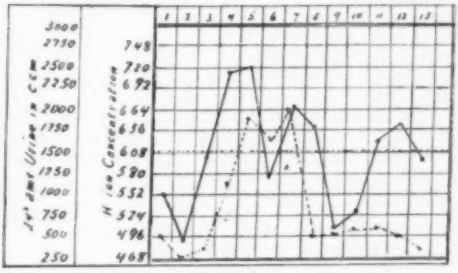

Chart 6.

Chart 5.—Case 5. Syphilitic aortitis. Aortic regurgitation; marked edema of legs. Made no improvement.

Chart 6.—Case 6. Arteriosclerosis. Weak dilated heart; double hydrothorax, marked edema of legs; marked improvement during period of diuresis with disappearance of edema then stationary. Discharged with compensation only partly restored.

acid excretory function might be suspected. It was believed that an abnormally large amount of acid in the urine would perhaps indicate the *overproduction* of acid in the organism, or partial failure of the protective mechanism.

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2. Henderson, L. J.: The Theory of Neutrality Regulation in the Animal Organism. Am. Jour. Physiol., 1908, xxi, 427.

The work to be reported in this communication is concerned only with urinary acidities in persons suffering from cardiac decompensation.

It is now well known that the true acidity of any solution must be expressed in terms of the concentration of ionized hydrogen; and that in complex solutions like urine, acidity determined in this way may give results which vary greatly from acidity determined by titration against

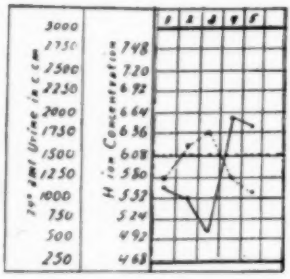

Chart 7.

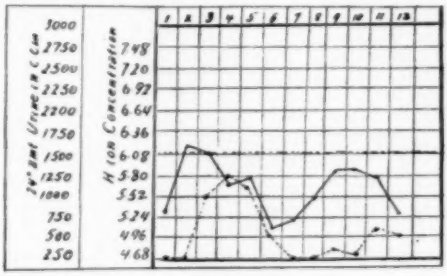

Chart 8.

Chart 7.—Case 7. Mild break. No edema. Rapid recovery.

Chart 8.—Case 8. Myocardial weakness. Marked dyspnea and orthopnea; weak, irregular pulse; no hydrothorax or ascites; very slight edema of legs. He improved rapidly at first, then became stationary, being unable to make any exertion without return of dyspnea.

alkali. In order to determine hydrogen ion concentrations in the clinical laboratory, some reasonably simple method had to be devised. This has been accomplished by two of us[3] and that method employed in this study.

For the convenience of the many clinicians who have not yet become familiar with the physico-chemical mode of expressing acidity, a brief statement will be added. Acidity is always thought of as the number of grams of ionized hydrogen in a solution when these are in excess of the hydroxyl ions. Since the

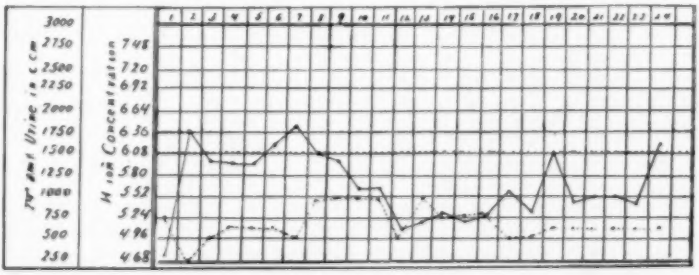

Chart 9.—Case 10. Aortic and mitral disease. Double hydrothorax; ascites; marked general anasarca; improved slowly; discharged with slight edema; otherwise no evidence of broken compensation.

3. Henderson, L. J., and Palmer, W. W.: On the Intensity of Urinary Acidity in Normal and Pathological Conditions. Jour. of Biol. Chemistry, 1913, xiii, 393.

purest water thus far obtained is slightly ionized and thus contains hydrogen ions (which, in the case of water, are in concentration equal to the hydroxyl ions) a solution, to be acid, must contain more hydrogen ions per liter than those resulting from the water, and at the same time, the hydrogen ions must be in excess of the hydroxyl ions. The ionization of hydrogen in water is actually 1/10,000,000 gram per liter at 25 C., or, otherwise expressed, 1×10^{-7}. Consequently the condition of acidity may be expressed as a formula thus:

$$(\overset{+}{H}) > 1/10{,}000{,}000\ N > (\overset{-}{O}H).$$

Neutrality is then

$$(\overset{+}{H}) = 1/10{,}000{,}000\ N = (\overset{-}{O}H).$$

And alkalinity

$$(\overset{+}{H}) < 1/10{,}000{,}000\ N < (\overset{-}{O}H).$$

$(\overset{+}{H})$ = the concentration of ionized hydrogen per liter expressed in the usual way.

$(\overset{-}{O}H)$ = the concentration of hydroxyl was per liter and 1/10,000,000 the concentration of the ions at the neutral point.

Since the ionization of water and of hydrogen and hydroxyl in solutions found in animal organisms is relatively so slight, the number of grams of hydrogen ions per liter is actually exceedingly small. It is consequently much more satisfactory to express the results as the logarithms of the number. Thus for water — 7.00 instead of 1/10,000,000. (As the numbers increase the acidity decreases; as the numbers decrease the acidity increases.) The minus sign is omitted for convenience.[4]

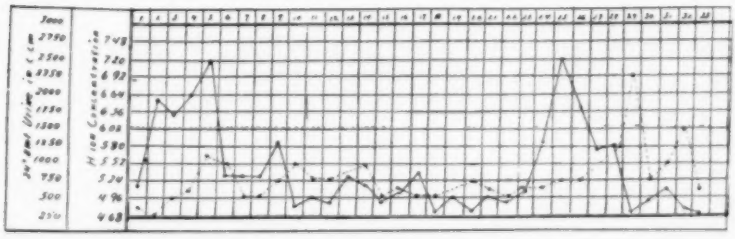

Chart 10.—Case 11. Mitral stenosis. Enlarged liver; edema of lungs; anasarca. During first period of diuresis there was not much clinical improvement, then came a period when condition was no better than at entrance. The second period of diuresis followed venesection and strophanthin intravenously, and was attended by marked clinical improvement, but the latter was only of several days' duration and the patient was finally discharged no better than at entrance.

The data here presented are derived from the daily examination of the twenty-four-hour specimens of urine of fifteen patients with "cardiac edema." The decompensation varied from the severest grade to the very mild type. The cases may be divided into two groups: (1) Degenerative, including arteriosclerosis with myocardial changes, and myocardial weak-

4. Further details concerning this physical-chemical conception of acidity will be found in Palmer and Henderson's paper in this issue p. 153: Clinical Studies in Acid Base Equilibrium and the Nature of Acidosis.

ness following interstitial nephritis. Cardiac failure occurring with glomerular nephritis was excluded. (2) Infectious, by which is meant injury to the valves dependent on microbic invasion with secondary failure of compensation. All active (malignant endocarditis) cases were excluded. The patients varied in age from 12 to 62 years.

The severe cases, eleven in number (Cases 1, 2, 3, 4, 5, 6, 8, 10, 11, 12, 14, see charts), had, on admission and up to the time when improvement began, a hydrogen ion concentration which averaged 4.90. The lowest single reading during this period was 5.30, the highest 4.70. The two cases with mild breaks in compensation (Cases 7 and 15, see charts) averaged 5.70, and the highest single reading was 5.30. In a study of the hydrogen ion concentration of the urines of one hundred and fifty

Table showing the hydrogen ion concentration of the twenty-four hour specimens of urine in eleven cases of severe cardiac decompensation, from admissions up to the time when definite improvement occurred. Cases 1 and 12 began to improve almost immediately after admission.

	Days											
Case	1	2	3	4	5	6	7	8	9	10	11	Average
1...	5.15	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	5.15
2...	4.85	5.00	.....	.....	.....	.....	.....	.....	.....	.....	.....	4.92
3...	5.00	5.15	5.15	5.15	5.00	5.00	5.00	4.85	5.00	4.85	4.70	5.00
4...	5.00	5.00	5.00	.....	.....	.....	.....	.....	.....	.....	.....	5.00
5...	5.15	5.00	5.00	5.15	5.15	5.30	5.30	5.15	5.00	5.15	.....	5.13
6...	4.85	4.85	4.70	4.85	.....	.....	.....	.....	.....	.....	.....	4.81
8...	4.70	4.70	4.70	.....	.....	.....	.....	.....	.....	.....	.....	4.70
9...	4.70	5.00	5.15	5.15	5.15	5.00	.....	.....	.....	.....	.....	5.02
11...	4.85	4.70	5.00	.....	.....	.....	.....	.....	.....	.....	.....	4.85
12...	4.85	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	4.85
14...	5.00	5.00	5.00	.....	.....	.....	.....	.....	.....	.....	.....	5.00

normal persons,[2] we have found the normal average to be 6.00. In less than 1 per cent. of these normal cases was the hydrogen ion concentration greater than 5.00, and in less than 10 per cent. was it greater than 5.30. Thus it is clear that persons with severe heart disease produce urine whose hydrogen ion concentration is distinctly above the normal.

If the hydrogen ion concentration is followed in reference to the clinical course of the cases (see figures), it is found that the former decreases as the individuals recover compensation (Cases 1, 2, and 14), and that in the individuals who have not improved markedly, the hydrogen ion concentration remains continuously high (Cases 3, 5, 8, 10, 11 and 12).

The next point to be considered is whether there is any relation between the edema and the hydrogen ion concentration. It is impossible to generalize on this question with the data at hand. However, certain facts seem to be true. At first sight, it would appear that hydrogen ion

concentration decreases when edema disappears; that is, when diuresis occurs. But in almost every instance general clinical improvement took place, coincident with the diuresis, so that these two factors cannot be separated; and consequently it cannot be said that diuresis occurred because hydrogen ion concentration was falling, or *vice versa*. On the other hand, the facts would seem to show that the hydrogen ion concen-

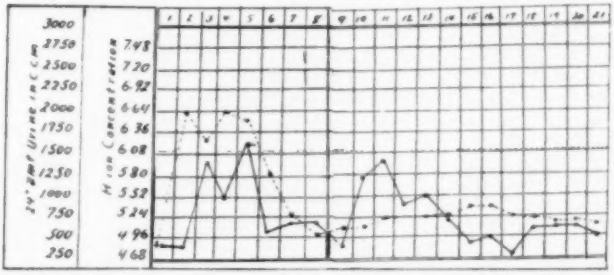

Chart 11.—Case 12. Mitral and aortic disease. Ascites; edema. Made marked improvement for first few days, then improvement ceased and the patient gradually failed to death.

tration depended more on the *severity* of the decompensation than on the *amount* of edema. For in Cases 2 and 8, in which the edema was very slight but in which the patients were very sick at entrance, the initial hydrogen ion concentration was as great as in persons who had massive edema (Cases 1, 4, 6, 10).

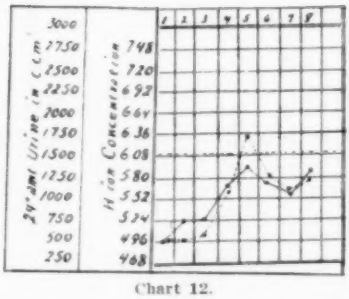

Chart 12.

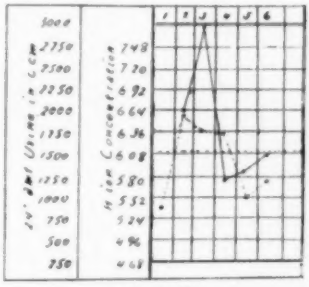

Chart 13.

Chart 12.—Case 14. Arteriosclerosis. Myocardial weakness; small ascites; moderate edema of legs. Made a slow but satisfactory recovery.

Chart 13.—Case 15. Mitral disease and break in compensation. Compensation restored in a week.

Furthermore, in Cases 4 and 6, in which a general temporary improvement occurred at the time of diuresis, with disappearance of edema and in which a stationary period then followed in which there was no edema, but compensation was only partly restored, it will be noted

that the hydrogen ion curve returned to its initial high level after the short period of temporary improvement and remained there even though the patients in the meantime had lost most of their edema.

In one case, No. 11, there were two periods of diuresis. During the first period there was no general improvement, and the curve for hydrogen ion concentration remained level. The second diuretic period was attended by marked clinical improvement of a few days' duration, and this time the hydrogen ion concentration decreased markedly.

CONCLUSIONS

1. The hydrogen ion concentration of the urine from individuals with severe cardiac decompensation is higher than the normal.

2. The hydrogen ion concentration follows the general clinical course, becoming normal when compensation is restored.

3. It could not be shown that there was any definite relation between the hydrogen ion concentration of the urine and edema in the cases studied.

329 Beacon Street.

CLINICAL STUDIES ON ACID BASE EQUILIBRIUM AND THE NATURE OF ACIDOSIS*

WALTER W. PALMER, M.D.,* AND LAWRENCE J. HENDERSON, M.D.

It is the purpose of this paper to present experimental evidence in favor of the view that varying grades of acidosis may exist, apart from diabetes and case in which beta-oxybutyric acid is produced, in many pathological conditions not previously suspected.

In health and in most diseases the very slightly alkaline reaction of the blood and body tissues is now known to remain practically constant.[1]

Walter,[2] long ago, showed that when an animal is killed by injecting an acid into the blood-stream, the blood apparently still remains alkaline. In disease, slight variations of reaction may occur, but death soon ensues if the change, as determined by the most accurate measurements, is appreciable.[3] In human pathology any change is always toward acidity. Such are the main facts regarding the variation of reaction of the blood.

In the body there is a constant production of acid substances as excretory products of metabolism. These acid bodies combine with base in the blood and are transported to the lungs and kidneys for excretion. Carbonic acid is excreted by the lungs. It devolves mainly on the kidney to excrete sulphuric acid, phosphoric acid, acetoacetic acid, beta-oxybutyric acid, etc.[4] If acid be produced more rapidly than it is excreted, or if the mechanism which regulates the excretion be at fault, the supply of base in the body being limited, a disturbance of the normal neutrality equilibrium may reasonably be expected to result.

The one important result of excessive production of acid, which has come to light, is the withdrawal of base from the body, with the attendant depletion of bicarbonate from the blood. The body is not known ever to produce base except ammonia to neutralize such acid. In the acidosis of diabetes the important factor is the excessive production of beta-oxybutyric acid. It is reasonable to suppose that there may be other conditions in which varying degrees of acid in excess of the normal in the

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* Submitted for publication May 1, 1913.

1. Henderson, L. J.: The Theory of Neutrality Regulation in the Animal Organism. Am. Jour. Physiol., 1908, xxi, 427.

2. Walter, Frederick: Untersuchungen über die Wirkung der Säuren auf den thierschen Organismus, Arch. f. exper. Pathol., 1877, vii, 148.

3. The investigations of H. Benedict show that the concentration of hydrogen ions in diabetic coma is about $1.5 \times 10 - 7$.

4. Henderson, L. J.: A Critical Study of the Process of Acid Excretion. Jour. Biol. Chem., 1911, ix, 403.

body fluids and tissues may exist, due to a disturbance in metabolism or insufficiency of the excretory mechanism. We have in truth succeeded in obtaining experimental evidence to support us in this view and the recent work of Sellards[5] has already revealed such cases. In this paper we are not concerned with particular acid substances or the cause for their existence, but only with the probability that a condition which may properly be regarded as acidosis in the broadest sense does frequently occur in unexpected circumstances.

Apart from diabetes, and cases in which beta-oxybutyric acid is formed, there are many conditions which clinically are frequently designated as "acid intoxications" or "acidoses." In the acute infectious diseases with high temperatures and severe toxemias, "acidity" is not infrequently mentioned. The acid factor in uremic conditions has long been suspected and work has been done to confirm this view.[6]

The scientific investigation of this subject has been considerable. In addition to the chemical analysis of the blood in conditions of acidosis, various means of determining changes in blood reaction have been employed. By means of the concentration cell measurements of the hydrogen ion concentration of blood, determinations of the tension of carbonic acid in the blood, as well as in the alveolar air of the lungs, titrations of the blood with acid or alkali to various indicator end-points, have been made in large numbers. Each of these methods of investigation possesses its peculiar utility, and on the whole such studies tend to support current ideas regarding acidosis.

The fact that in diabetes large quantities of alkali are necessary to make the urine alkaline has long been known. In acute rheumatic fever the same phenomenon is familiar to every practitioner. Sellards[5] has recently called attention to the fact that large amounts of sodium bicarbonate are necessary to influence the acidity of the urine in the uremia of Asiatic cholera and still later[7] in nephritis. Sellards also finds that the urine of normal individuals is rendered alkaline by the ingestion of sodium bicarbonate in smaller quantities than in the case of individuals with nephritis. In the nephritics he calls this phenomenon "increased alkali tolerance," but concludes that it may indicate a condition of acidosis. That large amounts of alkali are required to reduce the acidity of the urine in nephritis has also been observed by v. Hoesslin,[8] who

5. Sellards, A. W.: Tolerance for Alkalies in Asiatic Cholera. Phillipine Jour. Sc., 1910, v, 313.

6. Straub, H., and Schlayer: Die Urämie eine Säurevergiftung. München. Med. Wchnschr., 1912, lix, 569.

7. Sellards, A. W.: The Determination of Equilibrium in the Human Body between Acids and Bases with Especial Reference to Acidosis and Nephropathies. Bull. Johns Hopkins Hosp., 1912, xxiii, 289.

8. Hoesslin, V.: Ueber die Alhängigkeit der Albuminirie vom Säuregrad des Urin und über dem Einfluss der Alkalizufuhr auf Azidität, Albuminurie, Diurese und Chloridausscheidung sowie auf das Harnammoniak. Deutsch. Arch. f. klin. Med., 1912, cv, 147.

recommends alkali as a therapeutic measure in these cases. Recently Martin Fischer's[9] views on the cause and treatment of nephritis have attracted much attention. Mainly on theoretical consideration he advises the use of alkali in nephritis.

For more than a year past in our work on the acidity (hydrogen ion concentration) of the urine in nephritis we have found, as did Sellards and v. Hoesslin, that the ingestion of large quantities of alkali was frequently necessary before the urinary acidity could be diminished.[10]

In our work the effect of alkali in normal and a great variety of pathological cases has been studied by exact but simple quantitative methods. Throughout the entire investigation the method used for determining the acidity of the urine was the one described by us in a previous paper.[11]

For the convenience of clinicians the following modification is suggested:

Reagents:

1. N/10 Disodium phosphate.
2. N/10 Monopotassium phosphate.
3. N/5 Sodium acetate.
4. N/5 Acetic acid.
5. Two per cent. aqueous solution of sodium alizarin sulphonate.
6. Two per cent aqueous solution neutral red.
7. One per cent. alcoholic solution phenolphthalein.
8. Toluol.

Apparatus:

250 c.c. flasks (3286) obtained of Eimer and Amend, New York, have been found satisfactory. Medium sized test tubes of good, clear glass.

The disodium phosphate, $Na_2HPO_4 + 12H_2O$, is exposed to the air for a week whereby it loses 10 molecules of water of crystallization becoming $Na_2HPO_4 + 2H_2O$ which is stable. 17.8 grams to one liter makes a N/10 solution; 13.6 grams of KH_2PO_4 to one liter makes a N/10 solution; 27.2 grams of CH_3COONa to one liter makes a N/10 solution; N/10 CH_3COOH is made in the usual way by titration against a known alkali solution.

Standard solutions of known hydrogen ion concentration ($\overset{+}{H}$) are made as follows:

No.						$\overset{+}{H}$
1	0.5 c.c. N/10 KH_2PO_4	+ 240.0 c.c.	N/10 Na_2HPO_4	made up to 500 c.c. with H_2O		8.7*
2	0.5 c.c. N/10 KH_2PO_4	+ 60.0 c.c.	N/10 Na_2HPO_4	made up to 500 c.c. with H_2O		8.0*
3	5.0 c.c. N/10 KH_2PO_4	+ 25.0 c.c.	N/10 Na_2HPO_4	made up to 500 c.c. with H_2O		7.4†
4	5.0 c.c. N/10 KH_2PO_4	+ 11.5 c.c.	N/10 Na_2HPO_4	made up to 500 c.c. with H_2O		7.0†
5	2.25 c.c. N/5 CH_3COOH	+ 230.0 c.c.	N/5 CH_3COONa	made up to 500 c.c. with H_2O		6.7‡
6	5.75 c.c. N/5 CH_3COOH	+ 230.0 c.c.	N/5 CH_3COONa	made up to 500 c.c. with H_2O		6.3
7	11.50 c.c. N/5 CH_3COOH	+ 230.0 c.c.	N/5 CH_3COONa	made up to 500 c.c. with H_2O		6.0
8	23.00 c.c. N/5 CH_3COOH	+ 230.0 c.c.	N/5 CH_3COONa	made up to 500 c.c. with H_2O		5.7
9	57.5 c.c. N/5 CH_3COOH	+ 230.0 c.c.	N/5 CH_3COONa	made up to 500 c.c. with H_2O		5.3
10	115.00 c.c. N/5 CH_3COOH	+ 230.0 c.c.	N/5 CH_3COONa	made up to 500 c.c. with H_2O		5.0
11	230.00 c.c.	+ 230.0 c.c.	N/5 CH_3COOH	N/5 CH_3COONa	made up to 500 c.c. with H_2O	4.7

* Phenolphthalein.

† Neutral red and sodium alizarin sulphate.

‡ This and remainder of table sodium alizarin sulphate.

9. Fischer, Martin, H.: Nephritis, 1912, John Wiley and Sons, N. Y.

10. Our work was carried on for some time without knowledge of the work of Sellards.

11. Henderson, L. J., and Palmer, W. W.: Intensity of Urinary Acidity in Normal and Pathological Conditions. Jour. Biol. Chem., 1913, xiii, 393.

TABLE 1.—Response of the Kidney to Four Grams of Sodium Bicarbonate. Normal Cases.

No.	Time Alkali Given	$+_H$ Before Alkali	1		2		3		4		5		6		7		Diagnosis
			Time	$+_H$	Time	$+_H$	Time	$+_H$	Time	$+_H$	Time	$+_H$	Time	$+_H$	Time	$+_H$	
1	10.00	7.40	11.00	8.30	12.00	7.48	1.00	7.48	2.00	7.40	3.00	5.85	.....	.....	.....	.....	Normal.
2	10.00	5.85	11.00	8.30	12.00	8.15	1.00	6.85	2.00	6.00	3.00	7.20	12.00	7.00	*	7.48	Normal.
3	10.00	5.60	11.00	7.48	12.00	7.88	1.00	7.00	2.00	7.48	3.00	7.40	.....	.....	*	7.40	Normal.
4	10.00	5.15	11.00	7.20	12.00	7.20	1.00	6.70	2.00	7.00	3.00	7.20	.....	.....	*	5.70	Normal.
5	10.00	6.30	11.00	6.85	12.00	7.48	1.00	6.85	2.00	5.30	.....	.....	.....	.....	.....	.....	Normal.
6	10.00	6.00	11.30	7.20	.....	.....	.....	.....	2.00	7.48	.....	.....	4.00	7.40	.....	.....	Normal.

* Next a. m.

TABLE 2.—Response of the Kidney to Four Grams of Sodium Bicarbonate. Pathological Cases. Hourly Observations

No.	Time Alkali Given	$+$ H Before Alkali	1		2		3		4		5		6		7		Diagnosis
			Time	$+$ H	Time	$+$ H	Time	$+$ H	Time	$+$ H	Time	$+$ H	Time	$+$ H	Time	$+$ H	
1	10.00	5.40	11.00	7.48	12.00	7.20	1.00	6.50	2.00	5.70	3.00	5.70	12.00	5.70	*	5.70	Arteriosclerosis Weak heart.
2	11.00	5.70	12.20	7.20	1.05	5.85	2.20	7.00	6.30	7.48	.....	.....	.....	.....	*	7.48	Chronic neph. Hypertension.
3	10.00	5.00	11.00	5.15	12.00	7.20	1.00	7.20	.....	.....	.....	.....	.....	.....	.....	.....	Phthisis. Pneumothorax.
4	10.00	7.48	11.00	8.15	12.00	8.30	1.00	8.50	2.00	8.15	3.00	8.15	.....	.....	.....	.....	Incipient pul. tuberculosis.
5	10.15	5.30	11.05	6.15	12.00	6.70	1.15	5.70	2.15	6.50	3.15	6.70	11.00	6.00	*	6.15	Pernicious anemia.
6	10.00	5.30	11.00	7.20	12.00	7.20	1.00	5.85	.....	.....	.....	.....	.....	.....	.....	.....	Gastric cancer.
7	10.00	5.60	11.20	7.48	1.00	7.40	3.00	7.20	8.00	7.48	.....	.....	.....	.....	.....	.....	Acute tonsillar infection.
8	10.00	7.20	10.40	8.00	11.55	8.30	1.10	8.00	.....	.....	.....	.....	.....	.....	.....	.....	Acute endocarditis. Severe anemia.

* Next a. m.

TABLE 3.—Response of the Kidney to Four Grams of Sodium Bicarbonate. Pathological Cases. Two-Hourly Observations*

No.	Time Alkali Given	H^+ Before Alkali	1		2		3		4		5		7		Diagnosis
			Time	H^+	Time	H^+	Time	H^+	Time	H^+	Time	H^+	Time	H^+	
1	11.00	5.70	1.00	7.00	3.00	7.48	.....	.....	.....	.....	.....	.....	.....	.....	Acute nephritis.
2	9.00	5.00	11.00	7.40	1.00	7.40	3.00	7.40	6.30	7.20	.....	.....	†	6.70	Acute nephritis.
3	9.00	7.20	11.00	7.48	1.00	7.48	3.00	8.15	.....	.....	.....	.....	†	7.48	Acute nephritis.
4	9.30	4.70	11.00	6.70	1.30	6.70	3.30	5.70	6.20	6.85	.....	.....	†	5.40	Acute nephritis.
5	1.00	5.40	3.00	7.48	5.00	7.48	6.00	7.40	.....	.....	.....	.....	†	7.00	Acute appendicitis.
6	10.00	6.30	12.00	7.48	1.00	7.48	2.00	7.40	3.00	7.40	.....	.....	†	7.40	Chronic glomerulonephritis, hypertension.
7	10.00	5.85	12.00	6.50	1.00	6.00	3.00	6.30	.....	.....	.....	.....	.....	.....	Secondary anemia, unexplained
8	9.00	5.30	11.00	7.00	1.00	7.00	3.00	7.20	.....	.....	.....	.....	.....	.....	Pleurisy with effusion.
9	10.30	5.70	12.30	7.20	2.30	7.48	3.30	7.48	.....	.....	.....	.....	†	6.30	Empyema following lobar pneumonia.
10	9.00	4.85	11.00	5.40	1.00	6.70	3.00	5.70	.....	.....	.....	.....	.....	.....	Cardiac decompensation, aortic and mitral disease.
11	11.00	4.85	1.00	7.20	2.00	7.20	3.00	8.70	.....	.....	.....	.....	.....	.....	Cardiac decompensation. Mitral disease.
12	9.00	6.00	9.00	7.20	11.00	7.20	1.30	8.15	3.50	8.30	6.00	7.20	†	5.50	Cardiac decompensation. Mitral disease.
13	9.30	6.50	11.00	7.40	1.00	7.40	.....	.....	.....	.....	.....	.....	.....	.....	Acute bronchitis.
14	9.00	5.50	11.00	8.15	1.00	7.48	3.00	7.00	.....	.....	.....	.....	†	8.15	Acute bronchitis.
15	9.00	5.15	11.00	6.00	1.00	7.00	3.00	4.70	.....	.....	.....	.....	†	5.00	Influenza, bronchopneumonia
16	1.30	6.00	3.15	7.20	5.30	7.48	.....	.....	.....	.....	.....	.....	.....	.....	Hysteria.
17	10.00	6.00	12.00	7.20	3.00	7.20	4.30	7.20	.....	.....	.....	.....	.....	.....	Renal calculus.

* No test given on 6th.
† Next a. m.

TABLE 4.—Response of the Kidney to Four Grams of Sodium Bicarbonate. Specimens Collected at Irregular Intervals*

No.	Time. Alkali Given	+ H before Alkali	1		2		3		4		7		Diagnosis
			Time	+ H	Time	+ H	Time	+ H	Time	+ H	Time	+ H	
1	8.00	6.00	11.45	8.00	3.10	7.48	5.00	7.48	.....	.....	.....	.....	Normal.
2	11.00	5.40	4.00	7.00	5.30	5.50	6.30	5.50	.....	.....	.....	.....	Normal.
3	10.00	4.85	12.45	6.30	4.30	5.85	.....	.....	.....	.....	.....	.....	Arteriosclerosis with weak heart.
4	7.00	6.00	8.00	5.85	10.30	7.40	12.55	7.00	3.50	7.40	.....	.....	Acute infection.
5	10.00	5.70	11.00	7.40	3.00	7.40	5.30	5.40	.....	.....	†	5.40	Acute plumbism.
6	9.00	5.50	11.00	6.00	.....	.....	.....	.....	.....	.....	.....	.....	Carcinoma pancreas; metastasis in liver.
7	11.30	6.00	5.30	7.48	.....	.....	.....	.....	.....	.....	.....	.....	Acute rheumatic fever.
8	8.00	5.60	8.50	7.00	1.30	6.70	2.30	7.00	.....	.....	.....	.....	Carcinoma of bladder.
9	9.30	6.30	11.30	7.40	3.30	7.40	5.00	7.40	.....	.....	.....	.....	Gastric neurosis.
10	12.00	5.30	1.45	8.00	.....	.....	.....	.....	.....	.....	.....	.....	Gastric neurosis.
11	10.15	7.00	5.00	7.48	7.45	6.70	12.00	5.60	.....	.....	†	5.60	Duodenal ulcer.
12	9.00	7.00	11.00	8.00	.....	.....	.....	.....	.....	.....	.....	.....	Amebic dysentery.
13	9.30	6.50	10.30	7.40	3.40	6.30	5.00	7.48	.....	.....	.....	.....	Typhoid fever.
14	11.00	5.85	12.30	6.30	5.05	6.00	6.30	5.70	9.00	5.70	†	.....	Typhoid fever.
15	8.50	5.85	3.30	8.15	5.00	8.00	.....	.....	.....	.....	.....	.....	Gastric ulcer.
16	7.30	5.70	10.15	7.00	1.30	7.48	3.50	7.30	.....	.....	.....	.....	Hyperacidity.
17	11.00	5.85	3.00	6.00	4.20	8.00	.....	.....	.....	.....	.....	.....	Lung abscess.
18	1.10	5.30	3.45	7.20	6.45	7.40	.....	.....	.....	.....	.....	.....	Lung abscess.

* No tests given on fifth and sixth.
† Next a. m.

These standard solutions are put up in suitable bottles and a few cubic centimeters of toluol poured over each to prevent the growth of yeasts and molds.

In each of nine flasks is placed a 10 c.c. sample of each of the standard solutions, 3 to 11, the volume is made up to 250 c.c. with distilled water and 5 drops of the alizarin added. Care is necessary to have the concentration of indicator exactly equal in all cases. Ten c.c. of urine are next introduced into another flask and distilled water and indicator are added. The color of the diluted urine solution is next matched with one of the standard series.

If the reaction as thus measured falls between Solutions 3 and 5 a similar comparison is made using neutral red (5 drops) as an indicator. If the reaction is more alkaline than Solution 3, undiluted urine is matched in test-tubes against undiluted standard Solutions 1 and 2 using phenolphthalein (10 drops) as indicator. In case the reaction falls between the standard solutions, rough interpolation is made. The standard series of flasks containing the alizarin will keep, if corked, for three to four days in cool weather; in warm weather they should be made up fresh every other day. The solutions containing neutral red and phenolphthalein are made every time their use is required.

A word of explanation concerning the values thus determined is necessary. In physical chemistry acidity and alkalinity are expressed in terms of hydrogen or hydroxyl ion concentration. Pure water, H_2O, ionizes; that is, a few molecules break up into $\overset{+}{H}$ and OH ions in equal numbers, and we have neutrality. The amount of ionized hydrogen in water is exceedingly small, about 1 gram in 10,000,000 liters at 25 C. The phrase "hydrogen ion concentration" signifies the quantity of hydrogen ions present, expressed in terms of normality. Hence in water containing 1 gram of ionized hydrogen in 10,000,000 liters, or 0.000,000, 1 gram in one liter, the hydrogen ion concentration would be 0.000,000, 1 N, which may be written 1/ 10,000,000 N, or expressed more conveniently algebraically, $1x10^{-7}$ N. We have then for the definition of neutrality, acidity and alkalinity —

$(\overset{+}{H}) = 1x10^{-7} = (\overset{-}{OH})$ neutrality.

$(\overset{+}{H}) > 1x10^{-7} > (\overset{-}{OH})$ acidity.

$(\overset{+}{H}) < 1x10^{-7} < (\overset{-}{OH})$ alkalinity.

$(\overset{+}{H})$ stands for hydrogen ion concentration.

$(\overset{-}{OH})$ stands for hydroxyl ion concentration.

In normal blood, $(\overset{+}{H}) = 0.4x10^{-7}$, which is approximately the reaction of Solution 3.

In our work we have adopted the logarithmic notation. All logarithmics are of course negative, and for convenience the minus signs are omitted. The following table presents the conversion of the logarithmic

notation into actual concentration of ionized hydrogen. It will be seen that the smaller the logarithms the greater acidity.[12]

Log.	$\frac{+}{H}$	Log.	$\frac{+}{H}$
4.6	250×10^{-7}	6.4	4.0×10^{-7}
4.8	160×10^{-7}	6.6	2.5×10^{-7}
5.0	100×10^{-7}	6.8	1.6×10^{-7}
5.2	63×10^{-7}	7.0	1.0×10^{-7}
5.4	40×10^{-7}	7.2	0.63×10^{-7}
5.6	25×10^{-7}	7.4	0.40×10^{-7}
5.8	16×10^{-7}	7.6	0.25×10^{-7}
6.0	10×10^{-7}	7.8	0.16×10^{-7}
6.2	6.3×10^{-7}	8.0	0.10×70^{-7}

This method of studying urinary acidity is exceedingly simple, permitting numerous and accurate observations, and is well adapted to the range of reaction which may be encountered. The routine carried out in every case was the administration by mouth of 4 to 8 grams of sodium bicarbonate, at which time a specimen of urine was taken, as well as an hourly or two hourly specimen for several hours thereafter, the acidity being measured in each specimen. The acidity (hydrogen ion concentration) is recorded as the logarithm of the actual value. Hence, the smaller the logarithm the greater the acidity. (See note above.)

In suitable cases in which there was no response of urinary acidity to a normal dose of 4 grams of sodium bicarbonate, a sufficient quantity of the alkali was given to reduce the acidity of the urine. As soon as the effect of the alkali was apparent the sodium bicarbonate was omitted, the urine allowed to regain its former acidity and a second test was made with 4 grams of sodium bicarbonate. These observations were made in cases which showed very little clinical change during the course of the experiment.

As may be seen on examination of Tables 1, 2, 3, 4 and 5, the effect of small amounts of sodium bicarbonate is shown normally in the urine by a prompt and marked reduction of the acidity. This effect manifests itself soon after alkali intake. When hourly observations were made, in both normal and pathological cases, marked reduction of the acidity occurs in about an hour, the maximum effect in a little over one hour. In the cases when two hourly observations were made, marked reduction of urinary acidity was noted at the first observation after the alkali was taken. The average change in cases when there was a normal response to alkali was 1.67, which amounts approximately to a fifty-fold reduction in urinary acidity. After alkali intake the average degree of alkalinity reached was 7.60, which is slightly below the reaction of blood.

12. For detailed discussion of this subject see: Neubauer-Huppert: Analyse des Harns., 1910, i, 1. Neuberg, Carl: Der Harn, 1911, ii, 1396.

TABLE 5.—Response of the Kidney to Eight Grams of Sodium Bicarbonate

No.	Time Alkali Given	$\frac{+}{H}$ before Alkali	1		2		3		4		5		6		Diagnosis
			Time	$\frac{+}{H}$	Time	$\frac{+}{H}$	Time	$\frac{+}{H}$	Time	$\frac{+}{H}$	Time	$\frac{+}{H}$	Time	$\frac{+}{H}$	
1	10.00	5.70	11.00	8.50	12.00	8.30	1.00	6.50	2.00	6.50	3.00	7.40	.....	.....	Normal.
2	10.00	7.40	11.00	8.50	12.00	8.70	1.00	8.50	2.00	8.50	3.00	8.50	.....	.....	Normal.
3	10.30	6.70	6.30	8.50	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	Normal.
4	10.00	5.85	1.00	8.70	2.00	8.70	3.00	8.30	.....	.....	.....	.....	.....	.....	Normal.
5	10.00	6.70	11.00	7.48	12.00	8.70	1.00	8.50	2.00	8.70	5.00	8.50	.....	.....	Normal.
6	10.15	5.85	11.30	8.70	12.30	8.70	1.30	7.48	3.00	7.40	.....	.....	.....	.....	Chronic glomerulonephritis.
7	10.00	5.70	11.00	5.60	12.15	6.70	1.15	7.40	3.15	7.40	6.00	5.70	*	5.30	Hemophilia.
8	10.00	7.40	11.00	7.48	12.00	8.15	1.00	7.48	3.00	7.48	7.15	7.48	*	7.40	Acute tonsillitis.
9	10.00	5.60	11.00	7.40	12.00	7.20	1.00	7.00	2.00	7.00	3.00	7.20	*	5.85	Acute rheumatic fever.
10	10.00	7.40	11.00	7.48	12.15	8.15	1.15	8.00	3.15	7.48	9.00	6.30	*	7.40	Chronic glomerulonephritis; hypertension.
11	10.00	5.40	11.00	6.00	12.00	6.30	1.00	6.00	2.00	5.30	3.00	5.30	*	7.20	Cardiorenal disease.
12	3.30†	6.00	5.15	8.30	6.15	8.70	.....	.....	.....	.....	.....	.....	.....	.....	Cardiac decompensation; mitral disease.

* Next morning. † P. M.

In a few cases there was a delay of four to six hours before the maximum reduction in urinary acidity was reached. Because of the small number and variety of diseases in which this delay was observed, we make no attempt to explain it.

A great variety of diseases (Table 6) showed in certain cases no response to 4 grams of sodium bicarbonate. This lack of response to alkali occurred most frequently in patients with kidney disease. In comparing Table 6 with Tables 1, 2, 3, 4 and 5, it would appear, however, that the response or lack of response to alkali is not confined to any particular disease or group of diseases, but is more or less an individual phenomenon.

Several of the cases reported in Table 6 which showed no response on the initial 4 grams of sodium bicarbonate required varying amounts of alkali, 12 to 112 grams, before any effect on the acidity of the urine could be detected. With two exceptions after the urine had been allowed to regain its former acidity, 4 grams of sodium bicarbonate produced a prompt and marked effect on the urinary acidity, as it did in Tables 1, 2, 3, 4 and 5. This phenomenon we believe to be due to a need on the part of the body for alkali—to acidosis, in short. In kidney disease the prompt response to the second test made with the alkali would indicate that the kidney lesion *per se* was not a factor in the lack of response in the first instance. From unpublished data there seems to be no relation between the degree of acidosis and ammonia excretion in cases in which beta-oxybutyric acid is not produced.

All these observations lead us to the belief that the one well marked effect of over production of acid — decrease of sodium bicarbonate in the blood — is a frequent occurrence in many and various pathological conditions. As a result of our exact studies of urinary reaction and its change after the administration of alkali, together with what appears to us as the crucial experiment of the second administration of alkali immediately after the urine has become once more acid, we agree in the main with Sellards' conclusions, and believe that we have provided a more secure foundation for them. However, we regard his term "tolerance" for alkali, in the cases which do not respond to the administration of alkali by a diminution of urinary acidity, as unfortunate and misleading. We are strongly disposed to the view, which cannot be regarded as fully proved, that the phenomenon is merely due to retention of a normal constituent of the organism until its concentration — lowered by pathological processes — has been restored.

With these observations and considerations in mind we suggest that a condition of acidosis may be assumed to exist when the administration of a quantity of alkali equivalent to one liter of tenth normal solution fails to produce a diminution in the acidity of the urine. Hence the

TABLE 6.—No Response of the Kidney to Four Grams of Sodium Bicarbonate*

No.	Time Alkali Given	$+^{H}$ Before Alkali	1		2		3		4		5		6		7		Diagnosis
			Time	$+^{H}$	Time	$+^{H}$	Time	$+^{H}$	Time	$+^{H}$	Time	$+^{H}$	Time	$+^{H}$	Time	$+^{H}$	
1	11.00	5.60	2.15	5.85	3.00	6.00	11.45	5.15	.....	.....	.....	.....	.....	.....	.....	.....	Pleurisy with effusion.
2	10.00	5.00	1.00	5.30	3.00	5.30	5.30	5.40	.....	.....	.....	.....	.....	.....	.....	.....	Pleurisy with effusion.
3	11.00	5.30	3.25	5.40	6.00	5.40	11.30	5.50	.....	.....	.....	.....	.....	.....	.....	.....	Pleurisy with effusion.
4	11.00	6.15	12.00	5.85	1.00	5.70	2.00	5.60	3.00	5.60	4.00	5.70	6.30	6.15	.....	.....	Diabetes mellitus
5	11.00	5.30	12.00	5.40	1.00	5.15	2.00	5.30	3.00	5.30	4.00	5.40	12.10	5.70	.....	.....	Chronic glomerulonephritis.
6	10.00	4.85	2.00	4.85	4.00	4.85	12.00	4.85	.....	.....	.....	.....	.....	.....	.....	.....	Chronic glomerulonephritis.
7	10.00	5.30	11.00	5.30	12.00	5.60	3.00	5.30	.....	.....	.....	.....	.....	.....	†	5.50	Chronic glomerulonephritis.
8	12.30	5.00	5.00	5.00	7.15	4.85	10.50	5.15	.....	.....	.....	.....	.....	.....	†	4.85	Chronic nephritis
9	10.00	5.30	11.00	5.15	3.00	5.15	8.00	5.30	.....	.....	.....	.....	.....	.....	†	5.15	Arteriosclerosis; weak heart.
10	11.00	5.40	1.00	5.40	11.30	5.30	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	Arteriosclerosis; weak heart.
11	10.00	4.70	11.00	4.70	12.00	4.70	1.00	4.70	2.00	4.70	3.00	4.70	.....	.....	.....	.....	Hypernephroma.

12	9.00	5.15	11.00	5.30	1.00	5.00	3.00	5.15	8.00	5.15	.....	.....	.....	.....	†	5.30	Hypernephroma.
13	10.00	5.70	11.00	5.70	12.00	5.70	1.00	5.85	2.00	5.85	3.00	6.15	.....	.....	.....	.....	Pyelitis.
14	9.45	5.60	2.00	5.60	3.00	5.60	.....	.....	.....	.....	.....	.....	.....	.....	†	6.00	Acute nephritis.
15	10.00	5.15	11.00	5.15	12.00	5.15	1.00	5.15	2.00	5.15	3.00	5.15	6.00	5.15	†	5.15	Acute nephritis.
16	10.00	5.30	11.00	5.30	12.00	5.60	2.00	5.40	3.00	5.40	.....	.....	.....	.....	.....	.....	Pernicious anemia.
17	9.00	5.00	11.30	5.15	1.30	5.15	5.30	5.15	5.30	5.15	.....	.....	.....	.....	†	5.15	Pernicious anemia.
18	9.00	5.30	3.00	4.85	12.30	5.70	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	Gastric carcinoma.
19	9.30	5.50	11.30	5.85	1.30	5.50	3.30	5.30	.....	.....	.....	.....	.....	.....	.....	.....	Acute colitis.
20	9.30	5.40	11.30	5.40	1.30	5.40	3.30	5.40	.....	.....	.....	.....	.....	.....	.....	.....	Acute plumbism.
21	2.00	5.00	10.30	5.00	1.55	5.00	6.30	5.00	3.00	4.85	.....	.....	.....	.....	†	5.00	Lobar pneumonia
22	6.45	5.30	11.40	5.00	3.15	5.15	4.40	5.15	.....	.....	.....	.....	.....	.....	.....	.....	Ovarian cyst.
23	12.30	6.00	11.30	6.00	12.45	6.00	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	Typhoid fever.
24	11.10	5.15	1.00	5.50	3.00	5.50	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	Myelogenous leukemia.
25	10.00	6.30	10.30	6.30	3.30	5.85	6.00	5.70	.....	.....	.....	.....	.....	.....	.....	.....	Cardiac decompensation.
26	10.00	5.00	1.00	5.15	2.00	5.15	3.00	5.15	7.30	5.30	5.30	5.15	.....	.....	.....	.....	Unresolved pneumonia.
27	10.00	5.30	11.00	5.15	12.00	5.15	1.00	5.15	2.00	5.15	3.00	5.15	.....	.....	†	5.15	Chronic glomerulonephritis.
28	10.00	5.00	11.00	5.00	12.00	5.00	1.00	5.00	2.00	5.00	.....	.....	.....	.....	.....	.....	Chronic nephritis
29	11.20	5.00	2.00	5.15	6.30	5.15	9.00	5.40	5.00	5.30	.....	.....	.....	.....	.....	.....	Chronic nephritis

† Next morning.

* A reduction of less than five fold in urinary acidity was not considered a response.

TABLE 7.—TABLE OF AVERAGES

Number of Table	Average H^+ Before Alkali	Maximum H^+ Before Alkali	Minimum H^+ Before Alkali	Average Time Before Effect; Hrs.	Average Time of Maximum Effect; Hrs.	Average Change H^+ Noted	Maximum H^+ Change	Minimum H^+ Change	Average Low Limit H^+	Low Limit H^+ After Alkali	High Limit H^+ After Alkali	
1	6.05	5.15	7.40	1.1	1.2	1.65	2.45	0.90	7.70	8.30	7.20	Normal cases; hourly observations; 4 grams of alkali.
2	5.87	5.30	7.48	1.1	1.6	1.65	2.20	1.02	7.52	8.50	7.20	Pathological cases; hourly observations; 4 grams of alkali.
3	5.65	4.70	7.90	2.0	3.5	1.80	3.85	0.65	7.45	8.70	6.50	Pathological cases; two-hourly observations; 4 grams of alkali.
4	5.83	4.85	7.00	.....	.....	1.50	2.70	0.48	7.25	8.15	6.00	Pathological cases; irregular observations; 4 grams of alkali.
5	6.56	5.40	7.48	.....	.....	1.84	2.85	0.67	8.14	8.70	6.30	Normal and pathological cases; 8 grams of alkali.
6	5.30	4.70	6.30	.....	.....	.....	.....	.....	.....	.....	.....	Pathological cases showing no response to 4 grams of alkali.
...	*	.....	.....	1.4	2.1	1.67*	2.80	0.75	7.60*	8.30	6.63	General average in cases showing a response to alkali.

* Average obtained from all cases reported in previous tables.

condition of acidosis, so long vague and ill-defined, may at length be considered, whatever its origin and variety, as a depletion of bicarbonate from the blood, which would naturally reveal itself to the above test.[13]

We believe that these experiments also establish absolute indications for or against the therapeutic use of alkalies in many conditions. With a disturbance in the acid-base equilibrium in the body, which is probably due to an alkali depletion, it seems to us reasonable to attempt to restore the normal equilibrium by the administration of alkali. Certain facts concerning the use of alkali as a therapeutic measure should, however, be recognized. Alkali may be considered in the same catagory as water, good food, fresh air, and good nursing. As the result of many observations on the acidity of the urine in normal and pathological cases[11] we have found no case, without experimental interference, to have an acidity less than blood. In determining the extreme variations in acidity of the urine in normal and pathological individuals[14] we find that alkali given in amounts sufficient to push the reaction of the urine below that of blood a well marked albuminuria not infrequently results.

The gastro-intestinal disturbances from the use of too much alkali are well known. On the other hand there is no doubt that in many pathological conditions alkali in proper amounts makes the patients feel better. The choice of alkali depends on the individuals' taste and tolerance as well as the physicians' preference. Alkali should be given in large amounts (if sodium bicarbonate, 4 grams, three or four times a day) until the effect on the urinary acidity is noted, and thereafter in sufficient amounts to keep the reaction of the urine slightly more acid than blood.[15] The amount to accomplish this is frequently very small, not more than 4 grams daily. The same indications pertain to the use of alkali as a diuretic. In all of our cases with or without edema when there was a diuresis following the administration of alkali, the diuresis did not occur until the reduction of the acidity of the urine was established. It should be stated, however, that in some cases we have seen marked diuresis following the use of alkali; in others no diuresis.

When the therapeutic use of alkali is considered we recommend that at least the effect on the reaction of the urine be carefully watched or that the following procedure be followed:

1. Sodium bicarbonate, 4 grams, preferably between meals.

13. In all investigations on this subject it appears to be necessary to estimate with moderate accuracy the hydrogen ion concentration of the urine.

14. Henderson, L. J., and Palmer, W. W.: Extreme Variations of the Concentration of Ionized Hydrogen in Human Urine. Jour. Biol. Chem., 1913, xiv, 81.

15. We have found it convenient to estimate the amount of alkali given in terms of a tenth normal solution. Four grams of sodium bicarbonate makes approximately 500 c.c. of a tenth normal solution.

TABLE 8.—Cases in which There Was No Response to Four Grams of Sodium Bicarbonate

Alkali was given until urine became alkaline, then discontinued until the urine had regained its former acidity, when a second test with 4 grams of sodium bicarbonate was made.

Number.		Time Alkali Given	+ H Before Alkali	1		2		3		4		5		6		Remarks
				Time	+ H	Time	+ H	Time	+ H	Time	+ H	Time	+ H	Following Morning Time	+ H	
1	Before*	10.00	5.30	12.00	5.70	1.00	5.15	2.00	5.15	.....	.....	.....	.....	.....	.....	Pleurisy with effusion; 12 gm. alkali required to make urine alkaline.
	After†	10.00	5.30	11.00	7.48	12.00	7.48	1.00	7.00	2.00	7.00	3.00	7.00	.....	.....	
2	Before	11.00	5.40	1.00	5.40	11.30	5.40	.....	.....	.....	.....	.....	.....	.....	.....	Arteriosclerosis; 24 gm. alkali required to make urine alkaline.
	After	10.00	5.30	12.00	6.00	1.40	6.00	3.30	6.00	4.35	5.70	8.00	5.60	.....	.....	
3	Before	10.00	5.30	11.00	5.30	12.00	5.60	2.00	5.40	3.00	5.40	.....	.....	.....	.....	Pernicious anemia; 12 gm. alkali req'd to make urine alkaline.
	After	9.00	5.40	11.00	7.20	1.00	6.30	3.00	6.30	.....	.....	.....	.....	.....	.....	
4	Before	10.00	5.15	11.00	5.15	12.00	5.15	1.00	5.15	2.00	5.15	3.00	5.15	.....	.....	Acute nephritis. Marked ascites and edema; 112 grams alkali before urine became alkaline.
	After	9.00	5.30	11.00	6.30	1.00	7.20	3.00	5.85	10.00	7.48	1.00	7.48	5.00	7.48	
5	Before	1.00	5.30	3.25	5.40	6.00	5.40	11.00	5.50	.....	.....	.....	.....	.....	.....	Pleurisy with effusion; 36 grams alkali before urine became alkaline.
	After	6.00	5.70	8.00	5.60	2.00	7.00	6.30	7.00	.....	.....	.....	.....	7.00	7.00	
6	Before	12.30	5.00	5.00	5.00	7.15	4.85	10.30	5.15	.....	.....	.....	.....	7.00	4.85	Chronic nephritis; hypertension; no edema; 40 gm. alkali before urine became alkaline.
	After	11.15	5.15	3.25	6.30	9.30	7.20	.....	.....	.....	.....	.....	.....	6.30	7.20	

7	Before	2.00	5.00	10.30	5.00	1.55	5.00	.....	.....	.....	.....	.....	.....	7.00	5.00	Lobar pneumonia; 36 grams before urine became alkaline.
	After	9.15	5.40	10.30	6.70	3.15	6.70	.....	.....	.....	.....	.....	.....	.....	.....	
‡8	Before	9.00	5.15	11.00	5.40	1.30	5.30	3.00	5.30	.....	.....	.....	.....	.....	.....	Pernicious anemia; 24 gm. alkali before urine became alkaline.
	After	9.00	5.00	1.30	5.15	1.30	5.15	3.30	5.15	5.30	5.15	.....	.....	7.00	5.15	
‡9	Before	11.00	4.70	1.00	4.85	3.00	5.15	2.00	6.00	.....	.....	.....	.....	.....	.....	Gastric carcinoma; 24 grams alkali before urine became alkaline.
	After	9.00	5.15	11.00	5.30	1.00	5.30	.....	.....	.....	.....	.....	.....	6.45	6.30	
10	Before	10.50	5.50	3.25	5.50	5.25	5.50	6.40	5.50	.....	.....	.....	.....	6.00	5.50	First and second degree burns about face and chest. Slight trace of diacetic acid and acetone during experiment; 20 gm. alkali before urine became alkaline.
	After	10.00	5.50	11.30	7.40	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	
11	Before	9.00	5.15	11.30	5.15	1.00	5.30	4.00	5.15	6.40	5.15	.....	.....	7.00	5.30	Syphilitic nephritis. Marked ascites and edema; 24 grams alkali necessary before urine became alkaline.
	After	2.30	5.30	4.10	7.00	6.45	6.70	9.15	7.00	11.45	6.00	.....	.....	.....	.....	
12	Before	10.00	5.15	12.10	5.15	5.15	5.15	.....	.....	.....	.....	.....	.....	6.45	5.30	Chronic glomerulonephritis; no edema; 36 gm. alkali before urine became alkaline.
	After	10.00	5.30	12.00	5.85	5.40	5.30	.....	.....	.....	.....	.....	.....	.....	.....	

* Before: Observations *before* alkali had been given in amounts sufficient to influence urinary acidity.

† After: Observations *after* alkali had been given in amounts sufficient to influence urinary acidity and the urine allowed to regain its former acidity.

‡ Cases 8 and 9 failed to show the normal response when the second test was made, but this may be explained because of the five to seven days, which elapsed before the second test was made, thereby allowing time for a condition of acidosis to be reestablished

2. Determine the acidity on specimens of urine obtained at time of administration of the alkali and on single specimens passed within the following six or eight hours.

3. If marked reduction in urinary acidity occurs, alkali is not indicated and should not be given.

4. If no reduction is noted, alkali should be given until the desired effect on the urine is noted.

SUMMARY

1. A condition of acidosis appears to exist in a great variety of pathological conditions.

2. A simple clinical test is proposed for the detection of acidosis, whatever its origin.

3. A rational use and control of alkali in therapeutics is proposed.

STUDY XX: THE EFFECT OF DIURETIC DRUGS ON THE LIFE OF ANIMALS WITH SEVERE ACUTE NEPHRITIS *

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In a previous paper, Christian and O'Hare[1] have reported a detrimental effect of the diuretic drug, diuretin, in severe acute nephritis produced in rabbits by injections of uranium nitrate. Therefore, we were interested to study the effects of other drugs commonly employed by clinicians as diuretics, such as theocin, caffein, potassium acetate and spartein sulphate. The latter drug is included, as it is used by some clinicians as a diuretic, though not generally regarded as a diuretic in the same sense as the others.

Acute nephritis was produced in rabbits by uranium nitrate. Three and one-half mg. of uranium nitrate was dissolved in 1 c.c. of sterile distilled water, and the solution so made was given intravenously. Each animal received two doses twenty-four hours apart of 3.5 mg. of uranium nitrate per kilo of body weight. Uranium nitrate was employed to produce the nephritis because it causes a lesion bearing much resemblance to acute nephritis in man, and because previous work in this laboratory had made us familiar with its action. With these doses the nephritis is of a severe type, usually rapidly fatal.

The diuretic drugs were dissolved in sterile distilled water and were given intravenously, twice daily, the first dose twenty-four hours after the last (second) uranium injection. The drugs were given in amounts in proportion to those ordinarily given to an adult man weighing 170 pounds; that is, theocin[2] was given in 5 mg. doses per kilo of body

* This is one of a series of studies on experimental cardio-renal disease. Study I, Smith, Boston Med. and Surg. Jour., 1908, clviii, 696; Study II, Christian, Boston Med. and Surg. Jour., 1908, clix, 8; Study III, Christian, Jour. Am. Med. Assn., 1909, liii, 1792; Studies IV-XV, Christian, Smith and Walker, THE ARCHIVES INT. MED., 1911, viii, 468-551; Study XVI, Christian and O'Hare, THE ARCHIVES INT. MED., 1913, xi, 517; Studies XVII and XVIII, O'Hare, THE ARCHIVES INT. MED., 1913, xii, 49, 61; Study XIX, Christian and O'Hare, Jour. Med. Research, 1913, xxviii, 227.

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1. Christian and O'Hare: Study XVI. The ARCHIVES INT. MED.

2. Theocin sodium acetate (soluble theocin), F. Bayer & Co., Germany; Caffein, Merck & Co.; potassium acetate, obtained of local druggist; spartein sulphate, Merck & Co.

weight, caffein in 3 mg. doses per kilo of body weight, potassium acetate in 14 mg. doses per kilo of body weight, and spartein sulphate in 0.42 mg. doses per kilo of body weight. Because of the slight solubility in water of caffein, all the drugs were dissolved in equal and rather large amounts of water. Each solution was made so that the above dose of the drugs was contained in 4 c.c. of water; therefore, each animal received with the drug 4 c.c. of water per kilo of body weight.

Since such large amounts of water when given intravenously might be detrimental or otherwise in such cases, and secondly, since water itself is a good diuretic, the effect of the water was tested on other rabbits having a similar experimental nephritis. To them was given intravenously, twice daily 4 c.c. of sterile distilled water per kilo of body weight.

Normal rabbits were given water alone in similar amounts by the same methods, and also similar doses of the various drugs dissolved in equal amounts of water in order to determine the toxicity of the drugs. These animals serve as controls for the experiments with acute nephritis.

The effect of the diuretics, whether detrimental or beneficial, was judged by the length of life of the animals receiving them as compared with that of control rabbits which had a similar acute nephritis. The length of life in all cases was reckoned from the first injection of the uranium nitrate until the death of the animal.

FIRST SERIES OF EXPERIMENTS

In the first series (Table 1) 33 rabbits of varying weights were given two intravenous injections of uranium nitrate, 3.5 mg. per kilo of body weight, twenty-four hours apart. After an interval of twenty-four hours more a group of 7 of these habbits were given intravenous injections of theocin 5 mg. (in 4 c.c. of water) per kilo of body weight twice daily; 7 others were given similarly caffein 3 mg. per kilo of body weight; a third group of 7 potassium acetate 14 mg. per kilo of body weight, and a fourth group of 7 were given spartein sulphate 0.42 mg. per kilo of body weight; the remaining five rabbits were used as controls and were given no diuretics, these having had only the uranium nitrate. Of the 7 given theocin, 1 died in 3⅓ days, 4 in 4 and a fraction days, 2 in 5 and a fraction days. The average term of life of the group was 4 4/7 days, and the average number of doses of theocin per animal was seven. The group given potassium acetate ran a parallel course to those given theocin; 1 died in 3⅓ days, 3 in 4 and a fraction days, and 3 in 5 and a fraction days. The average length of life of this group was 4 4/7 days, and the average number of doses six per animal. The group given caffein lived slightly longer than the two previous groups. Three rabbits died in 4 and a fraction days, 3 in 5 days and 1 lived 6⅔ days; this latter animal brought the average length of life of the group up to 5 days, and the

TABLE 1.—Effect of Diuretic Drugs in Uranium Nephritis

Rabbit No.	Weight	Result	Drug	Mg. Drug	C.C. Fluid	No. Doses	Animals Dying on Successive Days After First Uranium Injection										Avg. No. Days
							3⅓ Days	4 Days	4⅓ Days	4⅔ Days	5 Days	5⅓ Days	5⅔ Days	6⅔ Days	8 Days	10 Days	14⅓ Days	
587	1210	died	Caffein ...	3.6	5.	6	..	..	1	..	..	..	..	..	..	..	..	.....
588	2210	died	Caffein ...	6.6	9.	6	..	..	..	1	..	..	..	..	..	..	..	.....
589	2110	died	Caffein ...	6.3	8.5	7	..	..	..	..	1	..	..	..	..	..	..	.....
594	2750	died	Caffein ...	8.1	11.	7	..	..	..	..	1	..	..	..	..	..	..	5
592	1900	died	Caffein ...	5.7	8.	10	..	..	..	..	..	..	..	1	..	..	..	.....
595	1750	died	Caffein ...	5.1	7.	5	..	..	1	..	..	..	..	..	..	..	..	.....
596	1750	died	Caffein ...	5.1	7.	7	..	..	..	..	..	1	..	..	..	..	..	.....
599	2650	died	Theocin ..	13.	10.5	7	..	..	..	..	..	1	..	..	..	..	..	.....
604	1300	died	Theocin ..	6.5	5.	6	..	..	..	1	..	..	..	..	..	..	..	4 4/7
613	2150	died	Theocin ..	10.5	8.5	6	..	..	..	1	..	..	..	..	..	..	..	.....
616	1360	died	Theocin ..	6.5	5.5	8	..	..	..	..	..	1	..	..	..	..	..	.....
618	1460	died	Theocin ..	7.	6.	5	..	..	1	..	..	..	..	..	..	..	..	.....
619	1350	died	Theocin ..	6.5	5.5	6	..	..	..	1	..	..	..	..	..	..	..	.....
620	1350	died	Theocin ..	6.5	5.5	4	1	..	..	..	..	..	..	..	..	..	..	.....
603	2160	died	Pot. Acet.	29.4	8.5	7	..	..	..	..	1	..	..	..	..	..	..	.....
605	2260	died	Pot. Acet.	30.8	9.	8	..	..	..	..	..	..	1	..	..	..	..	.....
610	1500	died	Pot. Acet.	21.	6.	4	..	1	..	..	..	..	..	..	..	..	..	4 4/7
614	2500	died	Pot. Acet.	35.	10.	4	..	1	..	..	..	..	..	..	..	..	..	.....
612	1800	died	Pot. Acet.	25.4	7.	6	..	..	..	1	..	..	..	..	..	..	..	.....
615	1260	died	Pot. Acet.	16.8	5.	4	1	..	..	..	..	..	..	..	..	..	..	.....
617	1550	died	Pot. Acet.	21.	6.	8	..	..	..	..	1	..	..	..	..	..	..	.....
590	2110	died	Spartein..	.88	8.5	8	..	..	..	..	..	..	1	..	..	..	..	.....
611	1350	died	Spartein..	.54	5.	8	..	..	..	..	..	..	1	..	..	..	..	.....
591	2010	died	Spartein..	.84	8.	11	..	..	..	..	..	..	..	1	..	..	..	6 for 6
593	2160	died	Spartein..	.88	8.5	7	..	..	..	..	1	..	..	..	..	..	..	1 lived
601	2400	died	Spartein..	1.	9.5	7	..	..	..	..	1	..	..	..	..	..	..	.....
598	1300	died	Spartein..	.54	5.	6	..	..	1	..	..	..	..	..	..	..	..	.....
597	1550	lived	Spartein..	.63	6.	11	..	..	..	..	..	..	..	..	..	..	..	.....
600	2550	died	.....	.....	.....	....	..	..	..	..	..	..	..	..	..	..	1	.....
602	2750	died	.....	.....	.....	....	..	..	..	..	..	..	..	..	..	1	..	.....
606	1850	died	.....	.....	.....	....	..	..	..	..	..	..	..	..	1	..	..	8 1/5
607	1600	died	.....	.....	.....	....	..	1	..	..	..	..	..	..	..	..	..	.....
609	2050	died	.....	.....	.....	....	..	..	1	..	..	..	..	..	..	..	..	.....

average number of doses per animal up to seven. The group of spartein animals lived a longer time both singly and collectively than did the previous three groups. One spartein rabbit lived 4⅓ days, 4 lived 5 and a fraction days, 1 lived 10 days, and 1 survived entirely, the injections being discontinued on the seventh day. The average length of life of this group, excluding the surviving rabbit, was 6 and a fraction days, and the average number of doses per animal was eight and two-sevenths. Of the 5 controls, 2 died in 4 and a fraction days, 1 on the 8th day, 1 on the 10th and 1 on the 14th day; an average length of life of 8¼ days.

In comparing the effect of these diuretic drugs on this series of 33 rabbits having an acute nephritis, it is evident that theocin and potassium acetate shortened the average life of the animal by almost one-half, and individually only two controls died before all the theocin and potassium acetate animals died. Caffein shortened the average length of life by 3 days, and all were dead before 3 of the 5 controls; still, caffein was not quite so detrimental as the previous two drugs. It may be said that spartein sulphate also was to some extent detrimental since 5 animals died during the time only 2 controls died; on the other hand, 1 spartein sulphate animal survived while none of the control animals survived. Spartein is much less harmful than the previous drugs used in this series, since only 1 died as early as 4⅓ days, and 2 died in 5⅓ and 5⅔ days, respectively, by which time all animals of the previous groups had died; in addition, 1 spartein rabbit lived 10 days and 1 survived entirely. Therefore, we may conclude that theocin, caffein and potassium acetate as given in these animals are very detrimental in acute experimental nephritis, in that they shorten materially the life of the animal. However, since these drugs were given in rather large amounts of water (4 c.c. per kilo) for intravenous injection, it might be considered that the water played an important part in the acute nephritis fatalities, either by making extra work for the kidneys, or by causing hemolysis of the red blood-cells and consequently hemoglobin poisoning or otherwise. On the other hand, with these large amounts of water, the drugs were greatly diluted and consequently less irritating. In order to determine the ill effects of any of these large amounts of water, the following series of animals were studied.

SECOND SERIES OF EXPERIMENTS

The second series of 8 rabbits (Table 2) were given an acute nephritis as in the previous series, and to 4 were given intravenously 4 c.c. of sterile distilled water per kilo of body weight twice daily, the other 4 being controls. Of the 4 given water, 1 died in 2⅓ days, 1 in 3⅓ days, and 2 died in 4 days, an average length of life of 5 5/12 days. Of the 4 controls, 1 died in 3⅓ days and 3 in 4 days, an average length of life

of 3 5/6 days. In comparing the animals individually, it is noted that in each group, 1 died in 3⅓ days, and 2 in 4 days; therefore, each group ran a parallel course for three animals. The fourth animal in the water group died over a day sooner than the fourth control. Consequently one might think that the water played an important part in one animal at least. Since the controls died so shortly after injection and all the water ones as well, we feel that these animals were unusually susceptible to uranium nitrate. That such is frequently the case is well known to those who have used uranium. This is substantiated by 19 control rabbits used in another experiment treated in the same way in which the average length of life was 6 days. Not being satisfied with the unusual early deaths of this series, this part of the work was repeated.

TABLE 2.—Effect of Sterile Water Injections in Uranium Nephritis

Rabbit No.	Weight	Result	Treatment	C.C. Fluid	No Doses	Animals Dying on Successive Days after First Uranium Injection					
						2⅓ Days	3⅓ Days	4 Days	4⅔ Days	5⅔ Days	Average No. Days
625	1370	died	Ster. H_2O	5.5	3	....	1	....	....	....	.....
626	1370	died	Ster. H_2O	5.5	4	....	....	1	....	....	3 5/12
628	1700	died	Ster. H_2O	7.	4	*	....	1	....	....	.....
627	2070	died	Ster. H_2O	8.	2	1	....	....	....	....	.....
621	1800	died	Controls	.....	.....	....	....	1	....	....	.....
623	2150	died	Controls	.....	.....	....	....	1	....	....	3 5/6
624	1650	died	Controls	.....	.....	....	....	1	....	....	.....
622	1570	died	Controls	.....	.....	....	1	....	....	....	.....

* This rabbit died immediately following last injection. Exit with convulsions, probably due to embolism.

THIRD SERIES OF EXPERIMENTS

A third series of 8 rabbits (Table 3) were used to repeat the work of the previous series. Of the 4 rabbits in this series receiving water, 2 died in 4⅔ days and 2 in 5⅔ days, whereas of the 4 controls, 1 died in 4⅔ days and 1 in 5⅔ days, and 2 entirely survived. This substantiates the results of the previous series, in that water in such large amounts intravenously does have in some cases a detrimental effect. But this effect is not constant, as was that with theocin, caffein and potassium acetate; neither was the effect so rapidly fatal since the rabbits receiving water all died in 4⅔ and 5⅔ days, whereas in the theocin series 6 of the 7 animals died inside of 4⅔ days, and in the caffein and potassium acetate

series 5 died inside of 5 days, and of these early deaths in each series 3 died on the 4th day. The spartein series parallels fairly closely the water series. Therefore, we may consider that water in such cases is detrimental to a certain extent in general, and may be very harmful in some cases. This substantiates more or less that diuretics (water being considered a diuretic) are detrimental in acute nephritis, but water is not as harmful as the diuretic drugs.

This brings up the question, are these drugs in themselves toxic? The next series was used to study the toxicity of the drugs and also of water alone in such large amounts when given intravenously to normal animals.

TABLE 3.—EFFECT OF STERILE WATER INJECTIONS IN URANIUM NEPHRITIS

Rabbit No.	Weight	Result	Treatment	C.C. Fluid	No Doses	Animals Dying on Successive Days after First Uranium Injection					
						2½ Days	3 ½ Days	4 Days	4½ Days	5½ Days	Average No. Days
639	2360	died	Ster. H_2O	9.5	6	....	....	....	1	....	.....
640	2060	died	Ster. H_2O	8.	6	....	....	....	1	....	5 1/6
641	2300	died	Ster. H_2O	9.	8	....	....	....	....	1	.....
642	2260	died	Ster. H_2O	9.	8	....	....	....	....	1	.....
635	2050	died	Controls	.....	.....	....	....	....	1	....	.....
636	1850	died	Controls	.....	.....	....	....	....	....	1	.....
637	2150	lived	Controls	.....	.....	....	....	....	....	....	.....
638	2600	lived	Controls	.....	.....	....	....	....	....	....	.....

FOURTH SERIES OF EXPERIMENTS

The fourth series consists of 12 normal rabbits, to 4 of which sterile water in doses of 4 c.c. per kilo of body weight was given intravenously, and to every 2 of the remaining 8 rabbits was given intravenously theocin, caffein, potassium acetate and spartein sulphate, respectively, using the same amounts of drug and water per kilo of body weight as was used in the first series of 33 animals which had an acute nephritis. All of these 12 rabbits survived 4 days of twice daily intravenous injections; that is, eight injections altogether, and all appeared normal and none the worse for the treatment, except that their ear veins were so thrombosed and injured that intravenous injections had to be discontinued. These rabbits received more intravenous injections than did the nephritis series and survived them in good condition, whereas the rabbits in the first series showed signs of failure usually a day before death, and

they died with seven or less injections. Consequently this series would seem to prove that the water alone and the diuretic drugs alone were not toxic when given intravenously in large doses to normal animals, over a reasonable length of time.

Histological study of the kidneys of all of the rabbits that died showed a severe grade of nephritis with but little variation in appearance in the different kidneys.

CONCLUSIONS

We feel that the following conclusions may be safely considered from this work and that they suggest a similar effect in man.

1. The diuretic drugs, theocin, caffein and potassium acetate definitely shorten the life of a rabbit having a severe acute experimental nephritis produced by uranium nitrate.

2. Theocin and potassium acetate gave quite parallel results and were slightly more harmful than caffein.

3. Spartein sulphate although not nearly so detrimental as the other drugs, however, did, in some cases, shorten the animal's life.

4. Water in large amounts is detrimental in some cases, possibly depending on the severity of the nephritis.

5. The diuretics alone in large doses and water alone in large doses when given intravenously to normal animals for a reasonable length of time do not shorten their life and probably are not toxic in themselves.

6. Diuretics are probably contra-indicated in severe acute nephritis in man, since in animals in such cases they shorten life.

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A CONTRIBUTION TO THE BACTERIOLOGY OF THE DUODENUM *

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Escherich,[1] in his study of intestinal bacteria in children, found that the small intestine during the intervals when only intestinal secretions are present is almost free from bacteria. Tissier[2] examined the duodenal fluid obtained at autopsy from children dying without digestive derangement. He found in his cultures numerous colonies of *B. coli* and the enterococcus of Thiercelin, together with other organisms in small numbers. Experimental work on animals and observations on men undergoing surgical operations by Cushing and Livingood[3] and by Kohlbrugge,[4] has shown that the small intestine becomes practically germ-free in the absence of food material, and speaks of autosterilization of the small intestine. The factors on which this autosterilization depend are not definitely known.

In adults the bacteria of the duodenum have been studied by Gessner[5] in eighteen cases in which autopsy was performed soon after death. Cases of digestive disturbance were excluded from the series. Gessner found an organism probably identical with *B. lactis aerogenes* in large numbers in eight cases, *B. coli* very frequently, two other types of bacilli, two kinds of staphylococci and one variety of streptococcus. Quantitative data were not obtained.

Hess[6] has studied the bacteria in the duodenal fluid of infants, employing a duodenal catheter to obtain the fluid. He found the staphylococcus to be the most common organism in the duodenal fluid of young infants. In examining fifteen cases he found only once the colon bacillus, or, indeed, any gas-producing organism, a result distinctly different from that obtained by previous study of the duodenal fluid obtained at autopsy. Hess suggests the use of the duodenal tube in studying the duodenal flora of adults, especially for the purpose of detecting the presence of typhoid bacilli.

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1. Escherich: Die Darmbakterien des Säuglings, 1886.
2. Tissier: Ann. de l'Inst. Pasteur, 1905, xix, 109.
3. Cushing and Livingood: Contributions to the Science of Medicine dedicated to W. H. Welch, 1900, p. 543.
4. Kohlbrugge: Centralbl. f. Bakteriol., 1901, xxix, 571; xxx, 10 and 70.
5. Gessner: Arch. f. Hyg., 1889, ix, 128.
6. Hess: Jour. Infect. Dis., 1912, ii, 71.

Our own observations were undertaken in order to perfect a technic by which duodenal fluid suitable for bacteriological study could be obtained from adults and to gain some knowledge concerning the microbes present in the duodenum in health and in gastro-intestinal disturbances.

TECHNIC OF OBTAINING DUODENAL CONTENTS

The duodenal tube consists of a small soft rubber tube, external diameter 3.5 mm., bearing at the lower end a perforated gold tip. This tube is sterilized by boiling in water for at least ten minutes. Before introducing the tube a gelatin capsule, which had been soaked for several days in 95 per cent. alcohol, is picked up with sterile forceps and tightly fitted over the gold tip of the tube. The tube with the gelatin capsule in place is dipped in shellac and dried several times until it is well covered. The coating of shellac prevents the gelatin capsule from being dissolved in the stomach. Several other methods were tried before the above was adopted; soaking the capsule in formaldehyd solution rendered it so hard that it would not dissolve in the duodenal juice; covering the tube with shellac without the use of the gelatin capsule filled up the perforations of the gold tip so effectually that the duodenal juice could not penetrate them; the use of thin rubber tissue over the tip of the tube and bursting it by inflation after the tube was in place was found to be impracticable.

After the tube is sterilized and the gelatin capsule in place and well covered with shellac, it is placed in the patient's mouth with instructions for him to swallow it. With the patient lying on his right side the tube is usually carried by the peristaltic waves of the stomach into the duodenum in about twenty minutes. Its position can be demonstrated either by the fluoroscope or by examination of the aspirated contents. The tube is usually given at half past 10 at night and the duodenal contents aspirated with a sterile glass syringe at about nine in the morning; then, with the tube in place, an Ewald test-breakfast is given and the duodenal contents aspirated one hour later. Patients sometimes object to taking the test-breakfast with the duodenal tube *in situ*. Occasionally, the tube is passed at 7 a. m., and the duodenal contents aspirated at 9 a. m. This method is more acceptable to the patient and seems to give as accurate results as the introduction of the tube at night.

The laboratory study of the duodenal fluids[7] consists of macroscopic and microscopic examination of the fluid, a direct microscopic count of the bacterial cells by the Winterberg method, quantitative inoculation of cultures on litmus-lactose-agar plates, ascitic-fluid-agar plates, shake-tube cultures according to the method of Veillon[8] in ascitic-glucose agar,

7. For a more detailed consideration of the methods see MacNeal, Latzer and Kerr, Jour. Infect. Dis., 1909, vi, 123 and 571.

8. Veillon and Zuber: Arch. de méd. exper., 1898, series 1, x, 517.

and cultures in fermentation tubes of glucose broth and lactose broth. In addition, a portion of the fluid is heated to 80 C. for ten minutes and inoculated in measured quantities into Veillon tubes of ascitic-fluid-glucose-agar and fermentation tubes of glucose and lactose broth for the detection of spores.

DETAILED RESULTS OF EXAMINATION

The results obtained with the first nine samples of fluid are omitted from this paper because of various faults in technic, relating more particularly to the removal of the material from the patient. There remain twenty-six fluids derived from twenty-four different patients, each of two patients having been examined twice.

Fluid 10 was removed from the patient R. L., 22 years old, who had suffered from alcoholic neuritis for the past year. He complained of severe pain in the abdomen and lower chest. His general nutrition was good and the analysis of his gastric contents after an Ewald test-breakfast was practically normal — total acidity 58 and free hydrochloric acid 28, expressed as c.c. of decinormal acid per 100 c.c. of gastric juice. Analysis of his urine and examination of his feces showed no signs of gastro-enteric disturbance.

The duodenal tube was introduced to a distance of 85 cm. from the incisor teeth at 10:30 p. m. November 7. The next morning an Ewald test-breakfast was given at 8 o'clock and at 9 a. m. 25 c.c. of duodenal contents were aspirated by means of a sterile glass syringe. Part of this, 15 c.c., was used for chemical study, which showed the presence of lipase, trypsin and bile. The remainder was subjected to bacteriological examination.

The latter specimen consisted of approximately 10 c.c. of a greenish-brown fluid with an abundant greenish-yellow sediment, amounting to about 2.5 c.c. in the bottom of the tube. It was odorless. Microscopically the sediment was found to consist of starch grains and a moderate number of yeast cells. The direct microscopic count showed 132,500 bacterial cells per milligram of fluid, most of them staining very feebly with the methylene blue. The plates of litmus lactose agar inoculated with 0.1 c.c. and 0.002 c.c. of fluid remained sterile after four days at 37 C. The plate of ascitic-fluid agar inoculated with 0.1 c.c. showed one colony, a Gram-positive, gelatin-liquefying coccus, Strain 1. The Veillon tubes and fermentation tubes showed no growth.

Fluid 11 was obtained from the patient C. L., aged 32, who had suffered from gastric ulcer for the previous six months. Soreness in the epigastrium and pain immediately after meals were the most prominent symptoms. He was a fireman by occupation and was well developed physically. His stomach contents showed total acidity, 75, and free hydrochloric acid 50. Examinations of the urine and feces were negative.

The duodenal tube was introduced at 10:30 p. m. Nov. 6, 1912, to the distance of 85 cm. from the incisor teeth. At 8 a. m., November 7, an Ewald test-breakfast was given and, one hour later, 15 c.c. of duodenal contents were aspirated by means of a sterile glass syringe. Part of this, about 8 c.c., was examined chemically. Its total acidity to phenolphthalein was 25; lipase and trypsin were present. The remainder was used for the bacteriological examination.

There were 7 c.c. of a greenish-yellow fluid containing a finely divided yellow sediment occupying the lower 2 c.c. The supernatant fluid was opalescent and odorless. Microscopic examination showed many starch grains, a moderate number of yeasts and crystals of cholesterin. The direct microscopic count showed 232,000 bacteria per milligram, most of them staining well with the

methylene blue, and 12,000 yeast cells per milligram. On the plates of litmus lactose agar 1,630 bacteria per c.c. developed into colonies, and on the ascitic-fluid agar 930 per c.c. Two of these colonies, one a micrococcus and one a bacillus, were transplanted and designated as Strains 2 and 3, respectively. The fermentation tubes gave positive growth, but there was no production of gas after five days. The Veillon tubes developed 1,200 colonies per c.c. Cultures inoculated with the heated fluid (spore material) gave no growth.

Fluid 12 was obtained from the patient G. H., female, 27 years old, who had suffered from duodenal ulcer with the usual symptoms for the previous year. Gastric analysis showed the total acidity to be 37 and free hydrochloric acid 10. The examinations of urine and feces were negative. The duodenal tube was introduced at 10:30 p. m., November 8, to the distance of 85 cm. At 9:30 a. m., November 9, without the ingestion of any food in the meantime, 13 c.c. of duodenal contents were aspirated. The chemical examination showed a total acidity of 8, and the presence of amylase, lipase and trypsin. A portion of the fluid was used for bacteriological examination.

The sample of fluid amounted to 3.5 c.c. It was orange yellow and clear except for a small amount of white flaky material. There was no perceptible odor. Microscopic examination of the sediment showed mucous threads, a few small flat crystals, apparently cholesterin, and a moderate number of bacteria. By the direct microscopic method 2,500 bacterial cells per milligram were counted. On the plates of litmus lactose agar 160 bacteria per c.c., and on the plates of ascitic-fluid agar 1,144 bacteria per c.c. developed into colonies. Three colonies were transplanted from the latter set of plates and the bacterial strains designated as 4, 5 and 6. The fermentation-tube cultures remained free from gas after eight days. The Veillon tubes of ascitic-fluid glucose agar brought to development about 1,200 bacteria per c.c. of fluid inoculated. The tubes inoculated with spore material remained sterile.

Fluid 13 was obtained from the patient S. W., 52 years old, suffering from a mild attack of cholecystitis, probably with gall-stones. The pains were general over the right hypochondrium. The patient was well nourished and in good physical condition. Gastric analysis showed total acidity 60, and free hydrochloric acid 30. Urine analysis was negative. The feces were very acid and contained much undigested material. The duodenal tube was introduced at 10:30 p. m., November 8, to the distance of 95 cm. from the incisor teeth. An Ewald test-breakfast was given at 9 a. m., November 9, while the tube remained in position, and at 10 a. m. 12 c.c. of duodenal contents were aspirated. The chemical analysis showed total acidity 10 (phenolphthalein), and the presence of lipase, trypsin and bile. The major portion of the fluid was used for the bacteriological examination.

There were approximately 10 c.c. of lemon yellow viscous fluid. Consistency was about that of the white of an egg. It was almost perfectly clear. Microscopic examination of the sediment showed a few yeasts and bacterial cells. By the direct microscopic count 600 bacteria per milligram were found. On the plates of litmus lactose agar and ascitic-fluid agar only 4 bacteria per c.c. developed into colonies. Two colonies were transplanted from the latter set of plates and the cultures designated as Strains 7 and 8. The fermentation-tube cultures showed no production of gas and the Veillon tubes remained sterile. The cultures inoculated with spore material also remained sterile.

Fluid 14 was obtained from the patient M. G. at 9:30 a. m., Nov. 11, 1912. The patient was a woman 36 year old suffering from a gastric ulcer for the previous two years. Radiographs showed an hour-glass contraction of the stomach. There was an area of exquisite tenderness over the epigastrium. The patient was under nourished and moderately anemic. She had lost several pounds in weight. No free hydrochloric acid was present in the gastric juice and the total acidity of it was 8. Examinations of urine and feces were negative. The duodenal tube was introduced at 10:30 p. m., November 10, to the distance

of 98 cm. No food was taken after 6 p. m., November 10. Between 9:15 a. m. and 9:50 a. m., November 11, 19 c.c. of duodenal contents were aspirated. The chemical analysis showed a total acidity of 8 (phenolphthalein) and the presence of amylase, lipase and trypsin. A part of the fluid was used for bacteriological examination.

The latter portion measured about 8 c.c. and consisted of an odorless, slightly smoky, yellow fluid containing a small amount of flocculent precipitate, distributed throughout the fluid. There was a little foam on the surface. Microscopic examination of the sediment showed large numbers of bacteria and, by direct microscopic count, 44,300 per milligram of fluid were found. On the plates of litmus lactose agar 9,200,000, and on the ascitic-fluid agar 3,900,000 bacteria per c.c. of fluid developed into colonies. Strains 9, 10, 11, 12 and 13 were taken from the former, and Strains 14, 15 and 16 from the latter set of plates. Glucose broth and lactose broth in fermentation tubes, inoculated with 0.1 c.c., 0.002 c.c. and 0.0002 c.c. was fermented with the production of 40 to 75 per cent. of gas, and in one tube there was 100 per cent. gas in the closed arm. The Veillon tubes developed colonies too numerous to count, most of them near the free surface. The tubes inoculated with spore material remained sterile.

Fluid 15 was obtained from patient T. B., male, aged 34, suffering for the previous ten years from duodenal ulcer. During this period he had free intermissions between attacks of intense pain and hemorrhage into the bowel. Gastric analysis showed total acidity 89, and free hydrochloric acid 70. There was no food retention in the stomach over night. Examinations of the urine and feces were negative. The duodenal tube was introduced at 10 p. m., November 11, to the distance of 83 cm. The last food was taken at 6 p. m., November 11. November 12 at 9 a. m. 9 c.c., at 9:10 a. m. 7 c.c., and at 10 a. m. 3 c.c. of the duodenal contents were aspirated. The chemical analysis of the first portion aspirated showed total acidity 5 (phenolphthalein) and the presence of amylase, lipase and trypsin. The second portion, aspirated at 9:10 a. m., showed a total acidity of 15 and again amylase, lipase and trypsin. The fluid withdrawn at 10 a. m. was used for the bacteriological examination.

The sample consisted of about 3 c.c. of almost clear, golden yellow fluid with a small amount of mucoid sediment. A slight odor suggestive of fresh fish was noted. Microscopic examination of the sediment showed very little formed material, a few irregular flat crystals, probably cholesterin, and some very finely divided vegetable material. Direct microscopic count showed 6,800 bacterial cells per milligram. On the plates of litmus lactose agar 10 bacteria per c.c., and on ascitic-fluid agar 70 bacteria per c.c. developed into colonies. Two colonies were transplanted from the latter series of plates and designated as Strains 17 and 18. All the fermentation-tube cultures remained free from gas. Of the Veillon tubes, one developed seven colonies and another a single colony. These tubes were each inoculated with 0.1 c.c. of the fluid. Tubes inoculated with smaller quantities remained sterile. Strains 19 and 20 were taken from the colonies in these tubes. The media inoculated with spore material remained sterile.

Fluid 16 was obtained from patient B. D., female, aged 24, suffering for the past six months from obstinate constipation with severe colonic pain and persistent headache. The radiograph showed the transverse colon to be prolapsed as far down as the pelvis. The patient had a pasty complexion but was well nourished. The gastric contents after an Ewald test-breakfast showed total acidity 50, and free hydrochloric acid 15. Examinations of the urine and feces were negative. The duodenal tube was introduced at midnight November 12 to the distance of 85 cm. At 9:30 a. m., without ingestion of food during the interval, 6 c.c. of duodenal contents were aspirated. The chemical examination showed total acidity 15 (phenolphthalein), and the presence of amylase, lipase

and trypsin. A small amount of the fluid was used for bacteriological examination.

The specimen consisted of approximately 1 c.c. of clear, golden yellow fluid with a small amount of sediment. Microscopic examination of the sediment showed bacilli and micrococci. By the direct microscopic counting method 148,250 bacteria per milligram of fluid were found. Plates inoculated with 0.1 c.c. and 0.002 c.c. of the fluid remained sterile, and the fermentation tubes remained free from gas. One of the Veillon tubes inoculated with 0.1 c.c. of fluid showed a surface growth, which was transplanted and designated as Strain 21. The tubes inoculated with spore material remained free from growth.

Fluid 17 was obtained from the patient F. S., male aged 43, suffering from intestinal and pulmonary tuberculosis. Four years previously he had had about 8 inches of the ileum removed for relief of a tuberculous stricture. Tuberculosis recurred in his intestine and became manifest in his lungs about three years later. The patient was anemic and had lost several pounds in weight. Gastric analysis showed total acidity 35, and free hydrochloric acid 20. Examinations of the feces and urine were negative. The duodenal tube was introduced at 10:30 p. m., November 13, to the distance of 83 cm. At 9:10 a. m., without ingestion of food in the interval, 12 c.c. of duodenal contents were aspirated. Chemical examination showed total acidity 13 (phenolphthalein), and the presence of amylase, lipase and trypsin. A small portion of the material was reserved for bacteriological study.

The sample consisted of approximately 3 c.c. of an almost perfectly clear, golden yellow, odorless fluid. Microscopic examination of the sediment showed micrococci and some unidentified débris. By the microscopic counting method 67,000 bacterial cells per milligram of fluid were found. Special microscopic examination for acid-proof bacilli was negative. Of the two sets of plates inoculated with 0.1 c.c. and 0.0002 c.c. of fluid, only the one plate of ascitic-fluid agar inoculated with 0.1 c.c. gave any growth. This showed three colonies, equivalent to 30 bacteria per c.c. of fluid. Two of these colonies were transplanted and the cultures designated as Strains 22 and 23. All the fermentation tubes remained free from gas. The Veillon tubes inoculated with 0.1 c.c. and 0.0002 c.c. each developed a single colony. These were transplanted and designated as Strains 24 and 25. The tubes inoculated with spore material remained sterile.

Fluid 18 was obtained from the patient J. F., male, aged 24, convalescing from an attack of influenzal pneumonia of two weeks' duration. The patient was fairly well nourished. Gastric analysis was not made. Examinations of the urine and feces were negative.

The duodenal tube was introduced at midnight November 13 to the distance of 63 cm. At 9:30 a. m., without the ingestion of any food in the meantime, 15 c.c. of duodenal contents were aspirated. The chemical examination showed total acidity 20 (phenolphthalein), and the presence of amylase, lipase and trypsin. A part of the fluid was used for bacteriological examination.

The sample for bacteriological study was approximately 3 c.c. of greenish-yellow, cloudy fluid, containing some flakes of mucus. Microscopic examination of the sediment showed only unidentified débris. By the direct counting method 125,000 bacterial cells per milligram of fluid were found. Special microscopic examination for acid-proof bacilli gave a negative result. On the plates of ascitic-fluid agar 3,400,000 bacteria per c.c. developed into colonies, and on the litmus lactose agar 2,300 per c.c. The fermentation tube of glucose broth inoculated with 0.1 c.c. produced a small bubble of gas and the lactose broth inoculated with the same amount produced 30 per cent. gas in the closed arm. Those inoculated with 0.0002 c.c. and with 0.1 c.c. of spore material produced no gas. The Veillon tubes, even those inoculated with 0.0002 c.c. of the fluid, developed colonies too numerous to count. Subculture Strains 26, 27, 28, 29 and 30 were

taken from the plates of ascitic-fluid agar and 31 and 32 from the plates of litmus lactose agar.

Fluid 19 was obtained from the patient C. F., female, 25 years old, suffering from neuritis, with indefinite pains over the abdomen. The patient had well developed hysteria. Gastric analysis showed total acidity 54, and free hydrocloric acid 30. Examinations of the urine and feces were negative.

The duodenal tube was introduced at 10:30 p. m., November 14, to the distance of 79 cm. The next morning at 9:30 a. m., without ingestion of food in the meantime, 10 c.c. of duodenal contents were aspirated. The chemical examination showed a total acidity of 10 (phenolphthalein), and the presence of amylase, lipase and trypsin.

The specimen for bacteriological study consisted of approximately 5 c.c. of odorless, cloudy, golden yellow fluid. Microscopic examination showed bacteria and yeasts amounting to 366,000 bacterial cells, and 1,320 yeast cells per milligram of fluid. Plates inoculated with 0.1 c.c. and 0.0002 c.c. remained free from colonies, except the plate of ascitic-fluid agar inoculated with 0.0002 c.c., on which two surface colonies developed. Although these probably represented contaminations they were transplanted and the strains designated as 33 and 34. The fermentation tubes and Veillon tubes inoculated with 0.1 c.c. and 0.0002 c.c. remained sterile.

Fluid 20 was obtained from the patient W. D., male, aged 18, suffering from a typhoid relapse. The duodenal tube was introduced at 10:30 p. m., November 14. The next morning the patient drank milk while the tube remained in position, and at 11 a. m. about 2 c.c. of duodenal contents were aspirated. It was used for the bacteriological examination.

There were approximately 2 c.c. of cloudy, greenish-yellow fluid, with a slight odor suggesting cheese. The direct microscopic count showed 775,000 bacteria per milligram of fluid. Plate cultures on ascitic-fluid agar brought to development 1,600,000, and on litmus lactose agar 1,100,000 bacteria per c.c. of fluid. From the former set of plates six colonies were transplanted and preserved as Strains 35, 36, 37, 38, 39 and 40. Seven alkaline colonies were transplanted from the plates of litmus-lactose agar and these strains were designated as 41, 42, 43, D 20(1), D 20(2), D 20(3) and D 20(4). The fermentation tube of glucose broth inoculated with 0.05 c.c. of the fluid showed 10 per cent. gas in the closed arm after four days and that of lactose broth showed 75 per cent. gas. The fermentation tubes inoculated with 0.0002 c.c. of the fluid and with 0.1 c.c. of spore material remained free from gas. The Veillon tubes inoculated with 0.05 c.c. and 0.0002 c.c. of the fluid developed colonies too numerous to count. The one inoculated with spore material (0.06 c.c.), showed no growth.

Fluid 21 was obtained from the patient J. K., female, aged 64, suffering from atrophic gastritis.[9] She complained of anorexia, vomiting and general weakness. Gastric analysis showed total acidity 10, and free hydrochloric acid 0. There were a few pus cells in the gastric lavage. Examinations of feces and urine were negative.

The duodenal tube was introduced at 10:30 p. m., November 15, to the distance of 99 cm. At 12:30 noon, November 16, without ingestion of any food in the meantime, 5 c.c. of duodenal contents were aspirated. Chemical analysis showed total acidity 10, and the presence of amylase, lipase and trypsin.

The portion for bacteriological study was approximately 5 c.c. of cloudy, golden-yellow fluid, with a flaky sediment. The microscopic findings and the direct bacterial count were not recorded. Plate cultures on ascitic-fluid agar brought to development 1,000,000 bacteria and on litmus lactose agar, 1,600,000 bacteria per c.c. of fluid. The usual fermentation-tube cultures of lactose broth were not inoculated with this fluid. In the fermentation tube of glucose broth

9. At the time of this examination the condition was diagnosed as gastric carcinoma. The subsequent history of the case was such that the diagnosis has been recognized as in all probability erroneous.

inoculated with 0.05 c.c. of the fluid, 30 per cent. of gas was produced. Similar tubes inoculated with 0.0002 c.c. of the unheated fluid and with 0.02 c.c. of spore material showed no gas. The Veillon tubes inoculated with 0.05 c.c. and 0.0002 c.c. of the fluid developed colonies too numerous to count; those inoculated with spore material remained sterile. Five subcultures taken from colonies on the plates of litmus lactose agar were designated as Strains 44, 45, 46, 47 and 48.

Fluid 22 was obtained from the patient M. G., at 12:15 noon, Nov. 16, 1912, the same patient from whom Fluid 14 had been obtained November 11. See page 181.

The duodenal tube was introduced at 10:30 p. m., November 15, to the distance of 99 cm. At 12:15 noon, November 16, about 3 c.c. of duodenal contents were aspirated. The patient had fasted while the tube remained in position. The specimen was used for bacteriological examination.

There were approximately 3 c.c. of clear, golden-yellow fluid. The results of the microscopic examination of the sediment and of the direct bacterial count were not recorded. On the plates of ascitic-fluid agar 85,000,000 bacteria per c.c. of fluid developed into colonies, and on the litmus lactose agar 31,000,000. Two colonies from the latter set of plates were transplanted and the strains preserved as 49 and 50, and three strains taken from colonies on the plates of ascitic-fluid agar were designated as 51, 52 and 53. Fermentation tubes of lactose broth were not inoculated. Fermentation tubes of dextrose broth, inoculated with 0.1 c.c. and 0.0002 c.c. of the fluid, produced 60 per cent. and 40 per cent. of gas, respectively. The Veillon tubes inoculated with 0.1 c.c. and 0.0002 c.c. of fluid developed colonies too numerous to count. The tubes inoculated with spore material showed no growth.

Fluid 23 was obtained from the patient R. E., female, aged 40, suffering from general enteroptosis and cystitis. Analysis of the gastric juice showed total acidity 27, and free hydrochloric acid 13. The urine contained much pus. Examination of the feces was negative.

The duodenal tube was introduced at 10:30 p. m., November 17, to the distance of 77 cm. The patient fasted until 9:30 a. m., November 18, when about 8 c.c. of duodenal contents were aspirated. The chemical examination showed total acidity 5 (phenolphthalein), and the presence of lipase and trypsin. A portion of the sample was used for bacteriological examination.

The specimen consisted of approximately 3 c.c. of cloudy, yellow fluid. By the direct microscopic counting method 124,000 bacteria per milligram of fluid were found. On the plates of litmus lactose agar 150 bacteria per c.c. developed into colonies and on the ascitic-fluid agar 550 per c.c. Three colonies were transplanted from the ascitic-agar plates and the strains designated as 54, 55 and 56. Four strains were taken from the litmus lactose agar. These were designated as 57, 58, 59 and 60. Fermentation tubes of glucose broth and lactose broth inoculated with 0.1 c.c. and 0.0002 c.c. of the fluid produced no gas. Veillon tubes of ascitic-fluid agar brought to development 200 bacteria per c.c. The tubes inoculated with spore material remained sterile. Two colonies were transplanted from the Veillon tubes and the cultures designated as Strains 61 and 62.

Fluid 24 was obtained from the patient E. H., female, aged 27, suffering from myasthenia gastrica and splanchnoptosis. This condition had persisted for several years. The patient was anemic but fairly well nourished. There was no gastric retention of food over night. Gastric analysis showed total acidity 56, and free hydrochloric acid 24. Examinations of the urine and feces were negative.

The duodenal tube was introduced at 10:30 p. m., November 17, to the distance of 85 cm. The patient fasted until 9 a. m., November 18, when 10 c.c. of duodenal contents were aspirated. Chemical analysis showed total acidity 5, and the presence of amylase, lipase and trypsin. A small portion of the sample was used for bacteriological examination.

This portion consisted of approximately 2 c.c. of perfectly clear, golden-yellow fluid. By the direct microscopic method 496,000 bacterial cells per milligram of the fluid were counted. Plate cultures on ascitic-fluid agar brought to development 130 bacteria per c.c. and cultures on litmus lactose agar brought to development 80 per c.c. Three of the colonies on the ascitic-fluid agar were transplanted and preserved as Strains 64, 65 and 66. One colony was transplanted from the litmus lactose agar and designated as Strain 67. Fermentation tubes inoculated with 0.05 c.c. and 0.0002 c.c. of the fluid remained free from gas. Veillon tubes brought to development 50 bacteria per c.c. of the fluid. The tubes inoculated with spore material remained sterile. One colony was transplanted from one of the Veillon tubes and designated as Strain 63.

Fluid 25 was obtained from the patient W. D., male, aged 42, suffering from hypertrophic cirrhosis of the liver. No gastric analysis was made. The urine contained much bile and the feces a diminished amount of bile pigment.

The duodenal tube was introduced at 10:30 p. m., November 18, to the distance of 88 cm. No food was taken until after 9:15 a. m., November 19, when 33 c.c. of duodenal contents were aspirated. Chemical analysis showed total acidity 3, and the presence of amylase, lipase and trypsin. The remainder of the fluid was used for bacteriological examination.

This portion consisted of approximately 23 c.c. of a clear golden-yellow fluid. Direct microscopic count showed 78,000 bacteria per milligram of the fluid. Plate cultures on ascitic-fluid agar brought to development 13,600,000 and on litmus lactose agar 2,900,000 bacteria per c.c. Four colonies were transplanted from the ascitic-fluid agar and designated as Strains 68, 69, 70 and 71, and two colonies transplanted from the litmus lactose agar were designated as Strains 72 and 73. Fermentation-tube cultures inoculated with 0.02 c.c. and 0.0002 c.c. of the fluid remained free from gas. The Veillon tubes developed colonies too abundant to count. The tubes inoculated with spore material remained sterile.

Fluid 26 was obtained from the patient J. A., male, aged 20, suffering from persistent epigastric pain. The liver and spleen were markedly enlarged and the cause of this enlargement was not ascertained. The condition was diagnosed as gastric ulcer. Gastric analysis showed total acidity 20, and free hydrochloric acid 5. Examinations of the feces and urine were negative.

The duodenal tube was introduced at 10:30 p. m., November 18, to the distance of 91 cm. No food was taken until after 9 a. m. on November 19, when 1 c.c. of duodenal contents was aspirated. This was used for bacteriological examination.

The sample consisted of approximately 1 c.c. of cloudy, golden-yellow fluid. Eight hundred sixty-four thousand bacterial cells per milligram were counted microscopically. Plate cultures on ascitic-fluid agar brought to development 850 and on litmus lactose agar 150 bacteria per c.c. of fluid. Two colonies were transplanted from the ascitic-fluid agar and designated as Strains 74 and 75, and two from the litmus lactose agar were designated as Strains 76 and 77. Fermentation tubes of glucose and lactose broth inoculated with the fluid remained free from gas. Veillon tubes brought to development 350 bacteria per c.c. of fluid. Tubes inoculated with spore material remained sterile. Three colonies were transplanted from the Veillon tubes and the subcultures designated as Strains 78, 79 and 80.

Fluid 27 was obtained from the patient B. B., male, aged 34, suffering from the recurrence of a chronic gastric ulcer. About one year before a gastroenterostomy had been performed because of stenosis of the pylorus due to the ulcer. He suffered intense epigastric pain which was somewhat relieved by vomiting. He had lost several pounds in weight and was anemic. Gastric analysis showed total acidity of 105 and free hydrochloric acid 90. Examinations of the urine and feces were negative.

The duodenal tube was introduced at 10:30 p. m., November 20, to the distance of 80 cm. At 10 a. m., November 21, before any food was taken, 60 c.c. of duodenal contents were aspirated. Chemical analysis showed total acidity 0, and the presence of amylase, lipase and trypsin. About a third of the fluid was used for bacteriological examination.

This portion consisted of approximately 20 c.c. of a very clear, golden-yellow fluid. Microscopic examination showed practically nothing but bacterial cells, which numbered 10,000 per milligram of the fluid. Plate cultures on ascitic-fluid agar brought to development 3,000, and on litmus-lactose agar 340 microbes per c.c. of fluid. Four colonies were transplanted from the ascitic-fluid agar and preserved as Strains 81, 82, 83 and 84. Fermentation-tube cultures of glucose broth and lactose broth inoculated with 0.1 c.c. of the fluid produced 60 per cent. and 25 per cent. gas, respectively. Similar tubes inoculated with 0.0002 c.c. of the fluid remained free from gas. The Veillon tubes developed enormous numbers of colonies and these were not counted. Tubes inoculated with spore material showed no growth.

Fluid 28 was obtained from the patient H. G., male, aged 29, suffering from asthenic gastritis. He complained of heaviness in his epigastrium and persistent bad taste. Gastric analysis showed total acidity 12, and free hydrochloric acid 0. Examinations of feces and urine were negative.

The duodenal tube was introduced at 10:30 p. m., November 20, to the distance of 85 cm. No food was taken before 9:50 a. m., November 21, at which time 8 c.c. of duodenal contents were aspirated. The chemical examination showed total acidity 5, and the presence of amylase, lipase and trypsin. Half of the fluid was used for the bacteriological examination.

There was approximately 4 c.c. of a fluorescent golden-yellow fluid. The microscopic examination was negative except for bacteria, of which 30,000 per milligram of the fluid were counted. Plate cultures on ascitic-fluid agar brought to development 120, and on litmus lactose agar 50 bacteria per c.c. Three colonies were transplanted from the ascitic-fluid agar and the strains designated as 85, 86 and 87. One subculture taken from the litmus lactose agar was designated as 88. The fermentation tube of lactose broth inoculated with 0.1 c.c. of the fluid produced 75 per cent. gas and that of glucose broth inoculated with a similar amount produced 5 per cent. Similar tubes inoculated with 0.0002 c.c. of the fluid remained free from gas. The Veillon tubes brought to development 150 bacteria per c.c. of fluid; one of these colonies was transplanted and preserved as Strain 89. Tubes inoculated with spore material remained sterile.

Fluid 29 was obtained from patient L. L., male, aged 23, suffering from a tuberculous pyelitis. No gastric analysis was made. The urine contained blood and pus. Examination of the feces was negative.

The duodenal tube was introduced at 10:30 p. m., November 24, to the distance of 90 cm. No food was taken while the tube was in position. At 9.15 a. m., November 25, 20 c.c. of duodenal contents were aspirated. The chemical examination showed total acidity 10, and the presence of amylase, lipase and trypsin. A small portion was used for bacteriological study.

This part consisted of approximately 3 c.c. of clear, smoky, golden-yellow fluid. One hundred fourteen thousand bacterial cells per milligram were counted by the direct microscopic method. Special microscopic examination for acid-proof bacilli gave a negative result. Plate cultures on ascitic-fluid agar brought to development 1,600,000 bacteria per c.c., and on litmus lactose agar only 80 bacteria per c.c. Four colonies were transplanted from ascitic-fluid agar and preserved as Strains 90, 91, 92 and 94, the number 93 seeming to have been inadvertently omitted. Fermentation-tube cultures remained free from gas. The Veillon tubes brought to development 1,000 bacteria per c.c. of the fluid. Two of these colonies were transplanted and designated as Strains 95 and 96. Tubes inoculated with spore material showed no growth.

Fluid 30 was obtained from the patient A. N., male, aged 48, suffering for several years from the symptoms of asthenic gastritis and from constipation, with much pain in the lower abdomen. Gastric analysis showed total acidity 14, and free hydrochloric acid 4. The urine contained a trace of indican. Examination of the feces was negative.

The duodenal tube was introduced at 10:30 p. m., November 25, to the length of 102 cm. No food was taken while the tube was in position. At 9:15 a. m., November 26, 10 c.c. of duodenal contents were aspirated. The chemical examination showed total acidity 5, and the presence of amylase, lipase and trypsin. A small portion of the sample was used for the bacteriological examination.

This portion was approximately 1 c.c. of clear, golden-yellow fluid with a very small amount of suspended material. Microscopic examination showed bacterial cells which numbered 33,400 per milligram according to the direct microscopic count. Plate cultures on ascitic-fluid agar brought to development 2,600 and on litmus lactose agar 2,400 bacteria per c.c. Two colonies were transplanted from the litmus lactose agar and the cultures designated as Strains 97 and 98, 99 and 100. Fermentation tubes of glucose and lactose broth inoculated with 0.05 c.c. of the fluid produced a small bubble of gas in each. Similar tubes inoculated with 0.0002 c.c. remained free from gas. The Veillon tubes brought to development a large number of bacteria, approximately 10,000 per c.c. of the fluid. Two of these colonies were transplanted and the subcultures designated as Strains 101 and 102.

Fluid 31 was obtained from patient K. H., female, aged 48, suffering from the symptoms of gastric neurosis and asthenic gastritis, with loss of weight and a feeling of heaviness in the epigastrium after meals. Gastric analysis showed total acidity 32, and free hydrochloric acid 13. Examinations of the feces and urine were negative.

The duodenal tube was introduced at 10:30 p. m., November 28, to the length of 80 cm. No food was taken while the tube remained in position. At 9:15 a. m., November 29, 2 c.c. of duodenal contents were aspirated, and this was used for the bacteriological examination.

The sample consisted of approximately 2 c.c. of a very viscid dark bile-stained fluid, cloudy and containing a great deal of mucus. Microscopic examination showed large flakes of mucus, crystals of cholesterin and some bile pigment. By the direct microscopic method 83,000 bacterial cells per milligram of fluid were counted. Plate cultures on ascitic-fluid agar brought to development 175,000 and on litmus lactose agar 41,000 bacteria per c.c. of the fluid. Four colonies were transplanted from the plates of ascitic-fluid agar, and the cultures designated as Strains 103, 104, 105 and 106. Two taken from litmus lactose agar were preserved as 107 and 108. Fermentation tubes of glucose broth inoculated with 0.05 c.c. and 0.0002 c.c. of the fluid produced 60 per cent. and 40 per cent. gas, respectively, and tubes of lactose broth inoculated with similar quantities produced 75 per cent. and 60 per cent. gas, respectively. The Veillon tubes brought to development about 100,000 bacteria per c.c. of the fluid. Two of the colonies were transplanted and preserved as Strains 109 and 110. The tubes inoculated with spore material gave no growth.

Fluid 32 was obtained from patient L. A., female, aged 38, suffering from an asthenic gastritis with indefinite pains in the stomach. The patient was convinced that she had a tapeworm but all examinations of the feces were negative. Gastric analysis December 3 showed total acidity 7, free hydrochloric acid 0; on December 5 total acidity 24, and free hydrochloric acid 16; on December 7 total acidity 8, and free hydrochloric acid 0. Examination of the urine was negative.

The duodenal tube was introduced at 10:30, November 29, to the distance of 95 cm. The patient fasted until 12:30 noon, November 30, when 2 c.c. of duodenal contents were aspirated.

The sample consisted of approximately 2 c.c. of golden-yellow fluid, quite clear. Microscopic examination was negative except for bacteria which numbered 6,800 per milligram of fluid, according to the direct microscopic count. Plate cultures inoculated with 0.05 c.c. and 0.0002 c.c. of fluid remained sterile, as also did the fermentation tubes and Veillon tubes inoculated with similar amounts.

Fluid 33 was obtained from patient P. M., male, aged 45, suffering from alcoholic gastritis and convalescing from delirium tremens. No gastric or fecal analyses were made. Examination of the urine was negative.

The duodenal tube was introduced at 10:30 p. m., December 1, to the distance of 83 cm. The patient fasted until 9:15 a. m., December 2, when 3 c.c. of duodenal contents were aspirated.

The specimen consisted of approximately 3 c.c. of clear greenish-yellow fluid with a little mucus in the bottom of the tube. Microscopic examination showed crystals of cholesterin, mucus and unidentified débris, besides the bacteria, which numbered 22,000 per milligram of the fluid. Plate cultures on ascitic-fluid agar brought to development 1,800,000 and on litmus lactose agar 2,700,000 bacteria per c.c. of fluid. Two subcultures taken from the ascitic-fluid agar were preserved as Strains 111 and 112, and three from the litmus lactose agar as 113, 114 and 115. One of the usual set of fermentation tubes, namely the glucose broth inoculated with 0.1 c.c. of the fluid, produced a small bubble of gas; the others remained free from gas. The Veillon tubes developed numerous colonies which were not counted. Two of them were transplanted and the strains designated as 116 and 117. Tubes inoculated with spore material gave no growth.

Fluid 34 was obtained from patient B. A., female, aged 30, suffering from myxedema and obesity. Analysis of the gastric juice December 14 showed total acidity 6, and free hydrochloric acid 0. The patient had about 30 pounds of superfluous fat. She complained of indefinite pains throughout the body. Examinations of the urine and feces were negative.

The duodenal tube was introduced at 7 a. m., December 9, to a distance of 116 cm. The patient fasted until 1 p. m., when 2 c.c. of duodenal contents were aspirated. This was used for bacteriological examination.

The specimen consisted of approximately 2 c.c. of a greenish-yellow fluid containing considerable mucus. Microscopic examination showed cholesterin crystals and 7,400 bacterial cells per milligram of the fluid. Plate cultures on ascitic-fluid agar brought to development 34,000 and on the litmus-lactose agar 21,000 bacteria per c.c. of the fluid. Strains 118, 119 and 120 were taken from ascitic-fluid agar and 121 and 122 from the litmus lactose agar. The usual set of fermentation tubes remained free from gas. The Veillon tubes brought to development a considerable number of colonies which were not counted. The examination of the spore material was omitted.

Fluid 35 was obtained from the same patient B. A., as Specimen 34.

The duodenal tube was introduced at 7 a. m., December 11, to a distance of 109 cm. The patient fasted until 12:30 noon, when 10 c.c. of duodenal contents were aspirated. This was used for bacteriological study.

The specimen consisted of approximately 10 c.c. of a fairly clear golden-yellow fluid. Microscopic examination showed considerable unidentified débris, and the count showed 2,800 bacterial cells per milligram of the fluid. Plate cultures on ascitic-fluid agar brought to development 1,000,000 and on litmus lactose agar 620,000 bacteria per c.c. of the fluid. The usual set of fermentation tubes remained free from gas. The Veillon tubes brought to development numerous colonies which were not counted. The tubes inoculated with spore material remained sterile. Strains 123, 124, 125, 126 and 127 were taken from colonies on the ascitic-fluid agar and 128, 129 and 130 from the litmus lactose agar.

TABLE 1.—DATA CONCERNING THE FLUIDS EXAMINED

Fluid No.	Diagnosis	Age	Sex	Gastric Acidity		Tube Length Cm.	Date	Amount c.c	Translucence	Color	Odor	Mucus	Food	Reaction to Litmus	Digestive State
				Total	Free HCl										
10	Neuritis	22	M	28	58	85	11/7	10	Cloudy	Lemon	0	0	‡	Acid	Ewald
19	Neuritis	25	F	30	54	79	11/15	5	Cloudy	Golden	0	0	0	Neutral	Fasting
34	Myxedema and obesity	30	F	0	6	116	12/9	2	Cloudy	Lemon	0	Much	0	.....	Fasting
35	Same case as No. 34...	30	F	0	6	.....	12/11	10	Clear	Golden	0	0	0	Neutral	Fasting
29	Pyelitis (tuberculous)	23	M	....	.....	90	11/25	3	Clear	Yellow	0	Viscid	0	Neutral	Fasting
18	Influenzal pneumonia..	24	M	....	.....	63	11/14	3	Cloudy	Lemon	0	Flakes	0	Alkaline	Fasting
17	Intestinal tuberculosis.	43	M	20	35	86	11/14	3	Clear	Golden	0	0	0	Acid	Fasting
13	Cholecystitis	52	M	30	60	95	11/9	10	Clear	Lemon	0	0	0	Acid	Fasting
20	Typhoid relapse	18	M	....	.....	91	11/15	2	Cloudy	Lemon	†	0	0	.....	Fasting
16	Prolapsed colon	24	F	15	50	.....	11/13	1	Clear	Golden	0	Slight	0	Alkaline	Fasting
23	Splanchnoptosis	40	F	13	27	77	11/18	3	Cloudy	Yellow	0	0	0	.....	Ewald
24	Splanchnoptosis	27	F	24	56	97	11/18	2	Clear	Golden	0	0	0	Neutral	Fasting
26	Asthenic gastritis	20	M	5	20	91	11/19	1	Cloudy	Golden	0	0	0	Acid	Fasting
28	Asthenis gastritis	29	M	0	12	91	11/21	4	Fluorescent	Golden	0	0	0	Neutral	Ewald
30	Asthenic gastritis	48	M	4	14	102	11/26	1	Clear	Golden	0	0	0	Neutral	Fasting
32	Asthenic gastritis	38	F	0	8	95	11/30	2	Clear	Golden	0	0	0	.....	Fasting
31	Gastric neurosis	48	F	13	32	80	11/29	2	Cloudy	Green	0	Much	0	.....	Fasting
33	Delirium tremens	45	M	....	.....	83	12/2	3	Clear	Lemon	0	Slight	0	.....	Fasting
25	Cirrhosis of liver.....	42	M	....	.....	88	11/19	23	Clear	Golden	0	0	0	Neutral	Fasting
27	Gastro-enterostomy	34	M	90	105	80	11/21	20	Clear	Golden	0	0	0	Alkaline	Ewald
15	Duodenal ulcer, healed (?)	34	M	20	89	83	11/12	3	Clear	Golden	0	Slight	0	Alkaline	Fasting
12	Duodenal ulcer.....	27	F	10	37	112	11/9	4	Clear	Orange	0	Slight	0	Alkaline	Fasting
11	Gastric ulcer	32	M	50	75	.....	11/7	7	Cloudy	Lemon	0	0	‡	Acid	Ewald
14	Gastric ulcer	36	F	0	8	98	11/11	8	Cloudy	Yellow	0	Slight	0	Neutral	Fasting
22	Same case as No. 14...	36	F	0	8	.....	11/16	3	Clear	Golden	0	0	0	.....	Fasting
21	Gastric carcinoma*....	64	F	0	10	99	11/16	5	Cloudy	Golden	0	.....	0	Neutral	Fasting

* The diagnosis was subsequently changed to atrophic gastritis in this case. † Cheesy. ‡ Much.

Another sample of duodenal fluid was obtained from this patient December 13. Chemical examination showed total acidity 5 (phenolphthalein), and the presence of amylase, lipase and trypsin.

The more important observations made in the series of examinations just described are summarized in Tables 1 and 2, in which the cases are grouped according to diagnosis, and in Table 3 in which the cases are rearranged in groups according to the number of colonies per cubic centimeter of fluid coming to development in the cultures.

TABLE 2.—DATA OF THE QUANTITATIVE BACTERIOLOGICAL EXAMINATIONS

Fluid No.	Direct Count Cells Per Cu. mm.	Cultures Inoculated with the Unheated Fluid*				
		Plate Cultures			Fermentation Tubes	
		Litmus Lactose Agar Colonies Per c.c.	Ascitic-Fluid Agar Colonies Per c.c.	Ascitic Glucose Agar, Tall Tubes Colonies Per c.c.	Dextrose Broth Gas Per Cent.	Lactose Broth Gas Per Cent.
10	132,000	0	10	0	0	0
19	367,000	0	0	0	0	0
34	7,400	21,000	34,000	.....	0	0
35	2,800	620,000	1,000,000	.....	0	0
29	114,000	80	1,600,000	1,000	0	0
18	125,000	2,300	3,400,000	†	‡	30
17	67,000	0	30	10	0	0
13	600	4	4	0	0	0
20	775,000	1,100,000	1,600,000	†	10	75
16	148,000	0	0	.....	0	0
23	124,000	150	550	200	0	0
24	496,000	80	130	50	0	0
26	864,000	150	850	350	0	0
28	30,000	50	120	150	5	75
30	33,000	2,400	2,600	10,000	‡	‡
32	6,800	0	0	0	0	0
31	83,000	41,000	175,000	100,000	50	68
33	22,000	2,700,000	1,800,000	†	‡	0
25	78,000	2,900,000	13,600,000	†	0	0
27	10,000	340	3,000	†	60	25
15	6,800	10	70	40	0	0
12	2,500	160	1,100	1,200	0	0
11	244,000	1,600	930	1,200	0	0
14	44,000	9,200,000	3,900,000	†	75	60
22	.....	31,000,000	85,000,000	†	50	.....
21	.....	1,600,000	1,000,000	†	30	.....

* Tubes of ascitic-fluid agar and fermentation tubes of dextrose broth and lactose broth inoculated with spore material (i. e. the duodenal fluid heated at 80 C. for ten minutes), remained free from growth in every instance.

† Very many. ‡ Slight.

TABLE 3.—THE NUMBER OF CULTIVABLE MICRO-ORGANISMS PER CUBIC CENTIMETER OF FLUID

Fluid No.	Colonies Per C.C.	Gas in Fermentation Tubes	Dominant Types	Gastric Acidity		Diagnosis
				HCl	Total	
Cases Showing 0 to 100 Colonies Per c.c. of the Fluid.						
10	0–10	0	Coccus	28	58	Neuritis.
19	0	0	.....	30	54	Neuritis.
13	4	0	Coccus	30	60	Cholecystitis.
16	0–10	0	Bacillus	15	50	Prolapsed colon.
17	0–30	0	Coccus	20	35	Intestinal tuberculosis.
32	0	0	.....	0	8	Asthenic gastritis.
15	10–70	0	Coccus	20	80	Duodenal ulcer (healed ?).
Cases Showing 100 to 10,000 Colonies Per c.c. of the Fluid.						
26	850	0	Coccus	5	20	Asthenic gastritis.
23	550	0	Yeast	13	27	Splanchnoptosis.
24	130	0	Yeast, bacillus and coccus	24	56	Splanchnoptosis.
28	150	+	Coccus	0	12	Asthenic gastritis.
30	10,000	+	Coccus	4	14	Asthenic gastritis.
27	3,000	+	Yeast	90	105	Gastro-enterostomy, ulcer(?).
11	1,630	0	Coccus and bacillus.....	50	75	Gastric ulcer.
12	1,900	0	Coccus	10	37	Duodenal ulcer.
Cases Showing 10,000 to 1,000,000 Colonies Per c.c. of the Fluid.						
34	34,000	0	Coccus	0	6	Myxedema and obesity.
35	1,000,000	0	Bacillus	0	66	Same case as 34.
31	175,000	+	Bacillus	13	32	Gastric neurosis.
Cases Showing More Than 1,000,000 Colonies Per c.c. of the Fluid.						
18	3,400,000	+	Coccus and bacillus.....	....	.....	Influenzal pneumonia.
29	1,600,000	0	Coccus	....	.....	Tuberculous pyelitis.
20	1,600,000	+	Coccus and bacillus.....	....	.....	Typhoid relapse.
33	1,800,000	+	Coccus	....	.....	Delirium tremens, convalescent.
14	9,000,000	+	Bacillus	0	8	Gastric ulcer.
22	85,000,000	+	Bacillus	0	8	Same case as 14.
21	1,600,000	+	Coccus and bacillus.....	0	10	Gastric carcinoma.*
25	13,600,000	0	Coccus	....	.....	Cirrhosis of liver.

* The diagnosis of this case was subsequently changed to atrophic gastritis.

NUMBER OF MICRO-ORGANISMS IN THE FLUIDS

Direct Microscopic Count.—The bacterial cells counted microscopically in the fluids varied from 600 to 860,000 per cubic millimeter, or from 600,000 to 860,000,000 per c.c. Evidently the bulk of these were actually dead; at any rate, the number of microbes brought to development in the cultures was only a small fraction of the number counted. These direct counts indicate that many bacterial cells gain entrance to the duodenum, doubtless after passing through the stomach, but otherwise they seem to have little or no significance.

The Colony Count.—The number of bacteria developing into colonies in cultures seems to bear a more definite relation to the gastro-intestinal condition. As will be seen in Table 3, those fluids showing less than 100 colonies per c.c., seven in number, were from cases showing, for the most part, normal gastric acidity. The free hydrochloric acid in these seven cases was 28-30-30-15-20-0-20 with an average of 20. The total acidity was 58-54-60-50-35-8-89 with an average of 51. The last two cases were somewhat abnormal and the findings in the sixth case are at least unusual when compared with the rest of this series.[10] Those fluids, from which between 100 and 10,000 colonies per c.c. were obtained, were derived from cases of gastro-intestinal disturbance. There were eight of these. The figures for free hydrochloric acid were, in order, 5-13-24-0-4-90-50-10, and for total acidity 20-27-56-12-14-105-75-37. There were three cases diagnosed asthenic gastritis, two as splanchnoptosis, one as gastric ulcer, one as duodenal ulcer and in one the operation of gastro-enterostomy had been performed. The three fluids, in which from 10,000 to 1,000,000 colonies per c.c. were obtained, were from cases diagnosed as myxedema with obesity, and gastric neurosis, respectively. In the former case the gastric juice showed free hydrochloric acid 0 and total acidity 6, and in the latter case hydrochloric acid 13 and total acidity 32. The fluids, from which a million or more colonies per c.c. developed, were from patients suffering from various diseases, most of them very ill. These results, as far as they go, would appear to indicate that normal duodenal fluid is practically free from living bacteria when food is absent, and that the number of colonies developed in cultures of the duodenal fluid is roughly an index of the digestive derangement. Where these are numerous the acidity of the gastric juice is often diminished, or there is other evidence of abnormality in the stomach or duodenum.

Bacterial Spores.—The cultures inoculated with spore material (the duodenal fluid heated to 80 F. for fifteen minutes) all failed to develop

10. Three analyses of gastric juice from this patient gave the following results: December 3, total acidity, 7; free HCl, 0; December 5, total acidity, 24; free HCl, 16; December 7, total acidity, 8; free HCl, 0. The duodenal fluid was obtained November 30.

colonies. The result suggests that the passage of bacterial spores through the stomach into the duodenum is not ordinarily a prominent factor in the bacteriological relationships in the intestine.

Gas-Forming Microbes.—The direct inoculation of fermentation tubes of glucose broth and lactose broth with the duodenal fluid, with subsequent incubation at 37 C., detected gas-producing organisms in ten of the twenty-six fluids. These ten fluids were from nine different patients, suffering from influenzal pneumonia (1), typhoid fever (1), asthenic gastritis (2), gastric neurosis (1), delirium tremens (1), recurrent ulcer after gastro-enterostomy (1), gastric ulcer (1) and atrophic gastritis (1). It would seem that gas production in these cultures takes place only when there is considerable disturbance of digestion. In some instances the gas was evidently produced by bacilli and in other instances by yeasts.

GENERAL NATURE OF THE MICRO-ORGANISMS ISOLATED

During the progress of the work, 130 colonies were transplanted and preserved for later study. Of these, twenty-two strains were lost before being studied; two others, Strains 21 and 34, were discarded because they were derived from fluids (No. 16 and No. 19) which gave rise to so few colonies that these were regarded as contaminations. Four of the strains derived from Case 20, those designated as D 20 (1), D 20 (2), D 20 (3) and D 20 (4), were identified as *B. typhosus* by their morphology, cultural characters and agglutination by a typhoid serum. The remaining 106 strains have been studied and grouped according to their morphology, reaction to the Gram stain, gas production in glucose broth and liquefaction of gelatin. The groups thus established are composed of various kinds of bacteria, but serve roughly to classify the organisms.

Gram-Positive, Non-Liquefying Cocci.—Forty-three of the 106 strains belong to this group, and organisms of this type were isolated from sixteen of the twenty-six fluids examined—fluids 12-13-15-17-18-20-21-23-24-25-26-28-29-31-33 and 34. The fluids from which organisms of this group were not isolated were ten in number, namely, 10-11-14-16-19-22-27-30-32-35. Four of these, 10-16-19 and 32, were approximately sterile fluids, and in the case of Fluid 11, only one subculture survived for study. Three of the remaining five fluids contained more than 500,000 cultivable micro-organisms per cubic centimeter, and the cocci of this group may have been easily missed though present. In the other two instances there were about 3,000 colonies developed per cubic centimeter of fluid. The strains transplanted, four in one instance and five in the other, did not include an organism of this group.

The fluids from which Gram-positive and non-liquefying cocci were isolated included two which were nearly sterile, namely, 13 and 17, and

this was the only type of microbe found in No. 13. In those cases in which the colonies numbered between 40 and 1,000 per cubic centimeter, organisms of this type constituted the bulk of the living flora. Here were included one case of healed duodenal ulcer, No. 15, two cases of splanchnoptosis, Nos. 23 and 24, two cases of asthenic gastritis, Nos. 26 and 28, and one case of renal tuberculosis. In one fluid, which produced about 20,000 colonies per c.c., No. 34, these cocci were dominant, as was also the case in one fluid showing 2,000,000 colonies per c.c., a convalescent case of delirium tremens, and in another showing 3,000,000 colonies per c.c., a case of advanced cirrhosis of the liver. In other instances in which abundant colonies developed, the dominant organism was something else, even though these cocci were found. These instances included one case of influenzal pneumonia, one of typhoid fever, one of gastric neurosis and one of asthenic gastritis. In the cases of ulcer these cocci were also forced into the back-ground by the dominance of other types. It would seem that bacteria of this group appear in the duodenal fluid in slight disturbances and are also present in more grave conditions, although they may be obscured by excess of other microbic types.

Gram-Negative, Gas-Forming Bacilli, which Liquefy Gelatin.—Nineteen of the 106 strains belong in this group. They were isolated from only five of the twenty-six fluids examined, and two of these were derived from one individual. A single strain of this type was isolated from the case of influenzal pneumonia, No. 18, in which the fluid produced 100,000 colonies per c.c. In the case of typhoid relapse, No. 20, two of the twelve strains isolated belonged to this group. One case, No. 21, diagnosed at the time as gastric carcinoma, seemed to have more living bacteria of this type than any other. Three of the five strains isolated from this fluid belong here. In this instance, 1,500,000 bacteria per c.c. of the fluid developed into colonies. The fourth case was diagnosed as gastric ulcer and the diagnosis confirmed at operation. Two fluids, No. 14 and No. 22, were obtained from this case November 11 and November 16. In the former 4,000,000 and in the latter 30,000,000 bacteria per c.c. developed into colonies, and these Gram-negative liquefying bacilli were the only kind represented in the thirteen subcultures. It would appear, therefore, that the bacteria of this type occur only in severe or very severe gastro-duodenal disturbance. The group may, perhaps, represent a single species. It is of unusual interest because of the fermentative properties, somewhat unusual for intestinal bacteria, and because of its abundance in some of the more severely ill patients.

Liquefying Gram-Positive Cocci.—Thirteen of the 106 strains belong in this group. Some of them were staphylococci and some appeared to be sarcines. They were found in eight of the twenty-four individuals. In only one case were they very numerous, and this was the case of

influenzal pneumonia, No. 18, in which the fluid produced 100,000 colonies per c.c., and three of the six strains isolated were found to be liquefying staphylococci. The remaining strains of this group were isolated from Fluid 10, which was almost sterile; No. 15, which produced forty colonies per c.c.; No. 12 and No. 30, which produced 1,100 and 2,500, and Fluids 25-31-34 and 35, which produced millions of colonies per c.c. These bacteria would appear, therefore, to be present in a very wide range of conditions, though probably not so commonly present as the cocci of the first group.

Yeasts.—Twelve of the 106 strains were cultures of yeast, and these were derived from five of the twenty-four patients examined. Fluids 20, 24 and 35 each furnished one strain of yeast. Two cases, No. 23 and No. 27, furnished all the rest. Fluid 23 was taken from a case diagnosed splanchnoptosis, and it produced 300 colonies per c.c. of fluid. Five of the nine strains isolated proved to be yeasts. Case 27 was one in which a gastro-enterostomy had been performed for the cure of ulcer. The fluid produced 4,000 colonies per c.c., and all four of the strains isolated proved to be yeasts.

Pleomorphic Bacteria.—Six of the 106 strains were made up of branched threads and spherical elements. These were at first regarded as mixed cultures, but plating failed to separate them, and it seems probable that the various forms present may belong to the same species. These forms occurred in only two specimens, No. 30 and No. 31. They constituted the dominant type in No. 30, in which 2,500 colonies developed per c.c. of fluid. This was a case of asthenic gastritis. In No. 31 only two of the eight strains isolated belong in this group. This case was diagnosed as gastric neurosis and 100,000 bacteria per c.c. of fluid developed in the cultures.

Gram-Positive Strepto-Bacilli.—Five of the 106 strains were made up of very regular bacilli joined together in long threads. Four of these failed to liquefy gelatin. Of these, two were isolated from Fluid 23 and two from Fluid 24, both diagnosed as splanchnoptosis, the number of colonies produced numbering 300 and 100 per c.c., respectively. The fifth strain in this group resembled the others in form and staining properties, but it caused liquefaction of gelatin. This was isolated from Fluid 31, from the case diagnosed as gastric neurosis.

Irregular Bacilli.—Bacilli irregular in shape and also in their reaction to the Gram stain are represented by four of the 106 culture strains. One of these was derived from Fluid 11, diagnosis of the case being gastric ulcer, and three were from Fluid 35, furnished by an individual suffering from myxedema and obesity.

Gram-Negative Gas-Forming Bacilli, which do not Liquefy Gelatin.—Only three of the 106 strains belong here. One was isolated from

Fluid 18, the case of influenzal pneumonia, and the other two from No. 31, a case of gastric neurosis. In each instance the cultures showed about 100,000 colonies per c.c. of fluid, and the colonies of this particular type were in the minority. This is, perhaps, one of the surprising features of this study, as it might have been expected that organisms of the *B. coli* group would be somewhat prominent members of the duodenal flora, in some conditions at least.

Gram-Negative Bacillus Producing Yellow Pigment.—One strain, isolated from Fluid 34 (case of myxedema and obesity), was a Gram-negative bacillus, morphologically like *B. coli*, which does not liquefy gelatin nor produce gas from dextrose, but does form a yellow pigment.

SUMMARY

1. It is possible, with proper care, to obtain a sample of the intestinal juice through the Einhorn duodenal tube sufficiently free from contamination for bacteriological study.

2. The normal duodenal fluid during a fast is almost free from living micro-organisms, although numerous bacterial cells are always visible on microscopic examination. The few living microbes obtained in cultures from such fluids are generally Gram-positive cocci.

3. In various gastro-intestinal disturbances, the number of cultivable microbes in the duodenal fluid is markedly increased. These organisms are of several different varieties, bacilli, cocci, yeasts and branching thread forms being represented in different cases.

4. In the one case of typhoid fever examined, *B. typhosus* was isolated from the duodenal fluid.

5. The bacteriological study of intestinal juice obtained in this way would seem worth while in cases of achylia gastrica with diarrhea and in cholecystitis. It also seems to us to be a promising field for investigation in those obscure diseases, the causation of which is sometimes ascribed to abnormal intestinal digestion.

6. It may also, perhaps, prove to be a procedure of value in the early diagnosis of typhoid fever, and in the detection of typhoid carries. We think it should be given a trial in this disease, as suggested by the previous work of Hess.

Acknowledgments are due to Miss Laura M. Stryker for assistance in the laborious microscopic and cultural examinations; to Dr. B. Lattin and Dr. W. G. Lough for personal attention to details in collecting the specimens from the patients, and to Prof. V. C. Myers for the data concerning the chemical examination of the duodenal fluids.

We also desire to express our obligation and thanks to Dr. Edward Quintard, Director of the Department of Medicine, for permission to use the clinical material.

Twentieth Street and Second Avenue.

THE FREQUENCY OF LOW POLYMORPHONUCLEAR LEUKOCYTE WITH HIGH LYMPHOCYTIC DIFFERENTIAL COUNTS *

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In the course of routine blood examinations made in Lane Hospital, San Francisco, it had been frequently noted that the polymorphonuclear leukocytes were below 60 per cent. in apparently normal individuals, and also that the lymphocytes ran over 40 per cent. in a sufficient number of apparently normal persons to make a differential count of somewhat doubtful value in the diagnosis of exophthalmic goiter, etc. In order to determine, if possible, the cause of these variations from the commonly accepted standard, the blood of one hundred normal individuals was examined.

These were selected with a view to including outdoor as well as indoor workers, females as well as males, and ages between 21 and 50; in short, to approximate as nearly as possible, the average individual appearing in the clinics or hospital. Among those examined were physicians, students and nurses at Lane Hospital, soldiers at the Presidio and such miscellaneous people as could be procured from time to time. All were in their usual state of health, care being taken to exclude those having recent illness. No other examination was made.

Various methods of spreading the blood were used. Ehrlich's method with coverslips, Da Costa's method with slides and Craig's methods with cigarette papers were tried and gave practically the same results. Two slides were made from each individual, two hundred cells being counted from each slide with an additional hundred in doubtful cases. Wright's stain was used. A total leukocyte count unfortunately could be made in only twenty of the hundred cases.

Average leucocytic count (20 cases) was 7,000.

Average differential count (100 cases)	Per cent.
Polymorphonuclear Neutrophils	56.55
Lymphocytes	37.45
Large Mononuclears and Transitionals	4.51
Eosinophils	.97
Basophils	.52
Polymorphonuclears in classes:	
(35) Soldiers	58.28
(22) Nurses	49.42
(18) Doctors and students	57.88
(25) Miscellaneous	58.96

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* From the Clinical Laboratory of Lane Hospital, Stanford University Medical Department.

Looking at these figures from another standpoint:

The polymorphonuclears in 93 of the total number were under 70 per cent.
The polymorphonuclears in 86 of the total number were under 65 per cent.
The polymorphonuclears in 59 of the total number were under 60 per cent.
The polymorphonuclears in 37 of the total number were under 55 per cent.
The polymorphonuclears in 22 of the total number were under 50 per cent.
The polymorphonuclears in 10 of the total number were under 45 per cent.

These results are markedly lower than those given in the text-books.

	Neutros. Per Cent.	Eos. Per Cent.	Basophils Per Cent.	Small Monos. Per Cent.	Large Monos. Per Cent.	Transitionals Per Cent.
Ehrlich and Lazarus (in Nothnagel)	70-72	2-4	.5	22-25	1	2-4
Naegeli (1912)	65-70	2-4	.5	25		
Pappenheim (1911)	73-75			20-22	2-8	
Krehl (1907)	70	1-4		28		
Cabot (in Osler)	60-70	.5-4	.1-.5	20-40 of which		1-10
Sahli (1911)	70-72	2-4	.5	22-25		2-4
Howell (1912)	60-75	2	.5	20-25	1	2-10
Da Costa	60-75	.5-5	.5	20-30	4-8	

On looking into the literature, among others who record low polynuclear counts, I find that Bunting[1] (1911) in Wisconsin got a neutrophil average of 54.6 per cent., large and small lymphocytes 35 per cent., transitionals 7.4 per cent. He believes that geographical location and altitude have some bearing on his results.

Guerrero and Sevilla,[2] as well as Chamberlain,[3] all work in the Philippines. Guerrero and Sevilla found polynuclears, 51 per cent., large and small 38.5 per cent.; Chamberlain, polynuclear average 54 per cent. Both believed that the tropical climate had an influence on the result.

Watkins,[4] in Arizona, says, in summing up the results of his counts:

> There is present in many individuals a considerable increase in the percentage of lymphocytes so that instead of being 30 per cent. they approximate 40, 45 or 50 per cent., or more. These increases are noted where the atmosphere is rarified by absence of moisture and by high temperature.

Hoxie,[5] in Missouri, gives low polymorphonuclear counts ranging from 67 to 49 per cent., which he attributes to auto-intoxication due to colonic stasis.

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1. Bunting: Am. Jour. Med. Sc., 1911, cxlii, 698.
2. Guerrero and Sevilla: Phillipine Jour. Sc., Series B, 1909, p. 277.
3. Chamberlain: Phillipine Jour. Sc., 1911, vi, 441, Series B.
4. Watkins, W.: Jour. Am. Med. Assn., 1911, lvii, 2129.
5. Hoxie: Jour. Am. Med. Assn., 1912, lviii, 1493.

Watkins (in same paper) gives counts on cured tuberculous patients with lymphocytes ranging from 40 to 70 per cent. Looked at from these standpoints, our results may have been influenced by

(a) Climate and geographical location.
(b) Previous tuberculous infections (I have no data on this matter).
(c) Auto-intoxication (extremely likely in a number of cases).
(d) The number of individuals examined is hardly large enough to eliminate chance.

To get a general idea of the range of the differential counts as they ordinarily present themselves in adults, the results of 500 counts were analyzed. These were taken from the medical, neurological, gynecological and surgical clinics and corresponding services at Lane Hospital—active tuberculous cases not being treated at either. The counts were made by Dr. Mylott of the Clinical Laboratory and by the intern staff (eight) of Lane Hospital, necessarily giving a more average technic.

Only those differential counts were included whose total leukocyte count was 10,000 or below.

The polymorphonuclears in 87 per cent. of the 500 were under 70 per cent.
The polymorphonuclears in 69 per cent. of the 500 were under 65 per cent.
The polymorphonuclears in 46 per cent. of the 500 were under 60 per cent.
The polymorphonuclears in 30 per cent. of the 500 were under 55 per cent.
The polymorphonuclears in 15 per cent. of the 500 were under 50 per cent.
The polymorphonuclears in 2 per cent. of the 500 were under 45 per cent.

These counts correspond fairly well with the results observed in supposedly normal people; considerably lower than the accepted normal. In fact, 75 per cent. "polymorphs" was so seldom reached that we were taught almost invariably to expect a numerical count greater than 10,000 when that number was obtained.

CONCLUSIONS

Our results and those reported in the literature would seem to indicate that low polymorphonuclear differential counts are very frequently observed in clinical work. Whether due to climate, geographical location, previous disease, condition of metabolism, or combination of these factors is not clear. At any rate, it becomes of importance for the clinician to remember the existence of this condition when he is tempted to give undue significance to a high lymphocytic count.

I am indebted to Dr. T. Addis for helpful suggestions, and to Capt. Hopwood, U. S. Medical Corps, for his courtesy in obtaining smears.

A CASE OF ACROMEGALY AND POLYGLANDULAR SYNDROME, WITH SPECIAL REFERENCE TO THE PINEAL GLAND*

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The term polyglandular syndrome is used by most writers to express the disturbed function of two or more glands of internal secretion. Cushing[1] has confined the term to cases in which it is impossible to tell which of the structures is primarily at fault. The combinations of the different glands is variable, as is the degree to which each is changed. From experimental data it is seen that the absence of the function of a normal gland (as for example, extirpation of that gland) leads to a definite symptom-complex in which some of the other glands participate. For instance, in the preadolescent eunuch, the impotency and the undeveloped secondary sexual characteristics are due to the immediate effects of the castration, while the growth of the long bones is thought to be due to a secondary hyperplasia of the anterior lobe of the hypophysis. Fischera[2] and others, have definitely shown that a secondary hyperplasia of the glandular part of the hypophysis, follows castration. In addition to the hypophyseal changes, Calzolarie, in 1898, and Henderson,[3] in 1904, respectively, have shown that in castrated rabbits and cattle the thymus may undergo hypertrophy and its involution be delayed.

The disturbance of function of one of the internal secreting glands also leads to a polyglandular involvement, as for example in exophthalmic goiter. In 135 cases of Graves' disease collected from the literature by Matti,[4] 74 per cent. were associated with a persistent thymus, while out of sixty cases reviewed by Capelle,[5] the thymus was enlarged in 79 per cent. of the cases. Since the symptom-complex of exophthalmic goiter has not been reproduced in animals, it is thought to be due to a perverted secretion of the thyroid gland. Just what the biologic disturbance is that underlies this abnormal secretion, has not been discovered.

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* Submitted for publication June 4, 1913.

* From the Department of Pathology, University of Chicago.

1. Cushing, H.: "The Pituitary Body and Its Disorders," 1912, p. 213.
2. Fischera, G.: "Ipofisi e Castrazione," Policlinico, Rome, 1910, xvii, 333.
3. Quoted by C. A. Parker: Surgery of the Thymus Gland, Am. Jour. Dis. Child., 1913, v, 89.
4. Matti, quoted by A. Crotti: Thymus Tracheostenosis and Thymus Death, Jour. Am. Med. Assn., 1913, lx, 571.
5. Capelle: Die Beziehungen der Thymus zum morbus Basedowii, Beitr. z klin. Chir., 1908, lviii, 353.

There are many other well known polyglandular syndromes, depending on either the ablation, the hyposecretion, the hypersecretion or the perverted secretion of certain glands. Prominent among these is acromegaly, supposedly due to a hyperplasia of the chromophil cells of the anterior lobe of the hypophysis. Acromegaly, or adult gigantism,[6] has never been reproduced in animals. It is accompanied by a polyglandular syndrome, involving one or more of the glands of internal secretion besides the hypophysis, which is always involved.

When in 1888, Marie[7] first pointed out the connection between hypophyseal strumas and acromegaly, he thought that a hyposecretion of the hypophysis was the probable cause; since then he has come to the conclusion that acromegaly is a perverted secretion of the anterior lobe. Others, namely, Silvestrini in 1894, Arnold in 1894, Petren in 1907, and Warda in 1901, have maintained that acromegaly and hypophyseal lesions are associated only accidentally, because they and others have performed autopsies on individuals dying from acromegaly in whom no pituitary lesion was found. These cases, however, must be accepted with reservation, for it is known that accessory nodules of hypophyseal tissue simulating anterior lobe tissue, may be found within the body of the sphenoid bone,[8] in the roof of the pharynx, and anywhere along the fetal craniopharyngeal duct. Lewis[9] reported a case of incipient acromegaly, in which the hypophysis was of normal size, yet presented histologically a proliferation of the chromophil cells of the anterior lobe.

Another theory of acromegaly points to an underlying biologic disturbance as the cause; this theory considers that the enlargement of the hypophysis is only one feature of a polyglandular syndrome, and that it is the result instead of the cause of acromegaly. Although no one disputes that some unknown disturbance underlies the gradual hypertrophy of the anterior lobe in acromegaly, clinical and anatomical evidence support the fact that bone changes follow instead of precede the hypophyseal hyperplasia.

Mussalango (1892), Tamburni (1894), Benda (1901), Modena (1903) and Fischer (1910), consider acromegaly the result of an hyperplasia or adenomatous condition of the anterior lobe of the hypophysis. An adenomatous struma is the type of tumor usually found in acromegalic individuals who come to autopsy.

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6. Cushing, H.: Quoting (a) Brissaud, E., and Meig, H.: Rev. scientif., 1895, iii, 330, and (b) Lannois, P. E., and Roy, P.: Etude biologique sur less geants, Paris, 1904. See note 1.

7. Marie, P.: L'Acromegalie, Nouv. Icon de la Salpêtrière, 1888, i, 173.

8. Erdheim, J.: Ubereinen Hypophysentumor von ungewöhnlichen Sitz., Beitr. z. path. Anat., 1909, xlvi, 233.

9. Lewis, D.: Hyperplasia of the Chromophil Cells of the Anterior Lobe of the Hypophysis as the Cause of Acromegaly, Bull. Johns Hop. Hosp., 1905, xvi, No. 170.

From birth on, the chromophil cells of the anterior lobe of the hypophysis increase; at 40 they are equal in number to the chromophobe cells of the same lobe; from 40 on the chromophil cells diminish in number so that in old age the chromophobes are again in preponderance as they are at birth (Lewis). During puberty, pregnancy and the climacteric, physiological hyperplasia of the chromophil cells is apparent. During the course of an acute disease, young patients often grow quite noticeably, a fact perhaps attributable to hyperplasia of the anterior lobe. Further, after castration, thyroidectomy and after pancreotomy, the anterior lobe is said to undergo enlargement. Benda,[10] in 1900, and Lewis,[9] in 1905, were the first to emphasize the fact that in the early stages of acromegaly, there is an hyperplasia of the chromophil cells of the anterior lobe, which apparently is the cause of the bone changes. Cushing, Fischer and others have adopted this view. Most investigators consider the chromophil cell as the most active cell in the gland. From clinical, anatomical and experimental findings, one is justified in assuming that these cells govern bone growth to a large extent. Massalongo and others have shown that if hypersecretion of the anterior lobe occurs in the early part of life before ossification of the long bones is completed, gigantism results; if, after ossification of the epiphyses, hyperplasia of the chromophil cells occurs, acromegaly results. In other words, acromegaly is adult gigantism.

It must be remembered that the chromophil cells composing hypophysial strumas, which are the cause of acromegaly, undergo degenerative processes early, in so far as the acidophil granules of these cells disappear entirely or in part. Small regions of necrosis occur, and cyst-like formations appear. It is probable that the so-called chromophobe strumas described in cases of acromegaly, as in most of Cushing's cases, are chromophil cell tumors from which the acidophil granules have disappeared through degenerative processes.

Assuming that increased secretion of the anterior lobe of the hypophysis is the cause of acromegaly and gigantism, one might suppose that feeding and injection experiments would produce overgrowth in puppies, while in old dogs some acral change would result. Neither of these results has been obtained. Caselli,[11] Cushing,[12] Aldrich,[13] Miller[14] and

10. Benda, C.: Beiträge zur normalen und pathologischen Histologie der menschlichen Hypophysis cerebri., Berl. klin. Wchnschr., 1900, 37, 1205.

11. Caselli, A.; Influence de la function de l'hypophysis sur le developpment de l'organism, Riv. sper. di frenatria, 1900, xxxvii, 176.

12. Cushing, H.: The Pituitary Body and Its Disorders, 1912, p. 11.

13. Aldrich, F. B.: The Feeding of Young White Rats with Dessicated Anterior and Posterior Lobes if the Hypophysis Separately, Am. Jour. Physiol., 1912, xxxi, 94.

14. Miller, J. L.: Quoted by Aldrich. See note 13

others, find that injection and feeding experiments not only do not increase the stature of animals, but retard their growth and stunt them; Caselli and Cushing worked with dogs, while Aldrich and Miller worked with white rats. Parisot[15] also found no noticeable changes with repeated injections of anterior lobe extracts into adult dogs, nor in puppies injected with small doses. With large injections in young animals, diminution in weight and size resulted. These findings suggest that the secretion of the chromophil cells of the anterior lobe is altered or perverted in acromegaly and gigantism, but by no means prove it, since injection and feeding of the gland or its extracts is crude and unreliable in comparison to the well-balanced mechanism of internal secretion.

Hyposecretion of the anterior lobe of the hypophysis is accompanied by bone changes exactly opposite to those of hypersecretion. It is generally known that young women suffering from exophthalmic goiter have delicate features and a small bony frame, as compared to the coarse features and large bones of the acromegalic or giant. Lewis and Benda both found a decrease in the eosinophilic type of chromophil cells of the anterior lobe of the hypophysis in patients dying from exophthalmic goiter.

The fact that heteroplastic tumors of the anterior lobe of the hypophysis occur without acromegaly or gigantism, is in accord with the findings of Benda, Lewis, Cushing, Fischer and others, relating to hyperplasia of the chromophil cells. Parisot[15] collected from the literature reports of seven cases of tuberculosis and of eight cases of gumma of the hypophysis, none of which had symptoms of acromegaly or gigantism. Many cases of sarcoma and of adenosarcoma accompanied by acromegaly have been reported in the literature, but in view of our present knowledge concerning the similarity of chromophil and of chromophobe strumas to sarcomas, reconsideration of the actual classification of these tumors must be made. Chromophil strumas and chromophobe strumas differ from sarcoma elsewhere, first, in so far as their course is longer, the average being three years; second, because they do not produce metastasis nor infiltrate the surrounding tissue, and third, as Mitchell and Le Count[16] have pointed out, the process of cell division is far less frequent than in sarcomas. In the case reported here, mitotic figures are extremely infrequent.

The following case of polyglandular syndrome was in a patient in the service of Dr. C. H. Lovewell of this city, through whose kindness and

15. Parisot, J.: Le rôle de la hypophysis dans la pathogenie de l'acromegalie, Rev. Neuroligique, 1910, xix, 277.

16. Mitchell and LeCount, E. R.: Report of a Necropsy in a Case of Acromegaly with a Critical Review of the Recorded Pathological Anatomy, New York Med. Jour., 1899, lxix, 517.

efforts an autopsy was permitted. The autopsy was performed by Dr. H. Gideon Wells and myself, on Nov. 20, 1912, about eight hours after the death of the patient.

CASE REPORT

Clinical History.—The patient died Nov. 19, 1912, at the age of 25. The parents state that he had moments of moodiness and forgetfulness when a boy. About one year before his death he noticed that his hands were getting larger; at this time he had occasional slight headaches. Two years prior to this, during his married life, he had been extremely passionate. About six months before his death he began to lose sexual desire, and two months later or four months before his death he became impotent. Mental symptoms of moodiness, forgetfulness and irritableness were becoming evident to his wife and also to the man for whom he worked as a driver. At about this time polyuria was marked; diaphoresis was so intense that during the night he would soak all of the bed clothes. One month before death headaches were frequent and very severe; his eyesight was becoming foggy, especially that of the left eye. November 13 he had a frightful headache and had to quit work. He was conscious until noon of November 18; he died at 4 a. m., November 19. It is thought by his wife that he became a little heavier during the few months prior to his death.

Autopsy Report.—The body is that of a man 6 feet and 3 inches long, well muscled and large boned. The weight is approximately 160 pounds; there is no abnormal amount of panniculus. The texture of the skin is normal. The face is large considered in its entirety, but the cheek bones, superciliary ridges, nose, and jaw, although of good size, are not unduly prominent. Nothing abnormal can be seen or felt about the head except that the right pupil is larger than the left one. The chest is a little flattened. The fingers and thumbs are noticeably enlarged, with the thickening which seems to be fibrous, more transverse than anterio-posterior. The left forefinger measures 75 mm. about the middle joint which is 10 mm. more than normal. The second joint of the second finger of the same hand measures 74 mm., which again is about 7 mm. more than normal. The corresponding joint of the middle finger of the right hand is 71 mm. in circumference. Both hands are perceptibly broadened. Each hand around the knuckles measures 211 mm. There is no apparent enlargement of the feet. The toes are in extreme extension. The superficial lymph-nodes are all slightly enlarged. The costochondral junctions are not swollen.

The abdominal cavity is free from fluid. There are a few fibrous adhesions about the gall-bladder and the spleen. The fat in the omentum, as elsewhere, is scanty. The liver extends three fingerbreadths below the costal margin on the right side. The retroperitoneal lymph-nodes and those in the mesentery are markedly enlarged; the largest mesenteric lymph-node measures 20 x 15 x 6 mm. Those lymph-nodes about the pancreas are even larger. The pleural and pericardial cavities are normal. The lymph-nodes in the mediastinal and in the cervical regions are enlarged and quite firm; on sectioning, their surfaces are reddish-gray in color. Nothing of importance is found in the trachea, lungs, esophagus or stomach. The intestines are full of fluid; the solitary follicles and Peyer's patches are enlarged.

The thyroid gland is about twice its normal size, weighing 65 grams, and contains a large amount of colloid evenly distributed throughout. An accessory lobule, 12 x 15 x 10 mm. is found behind and attached closely to the right lobe.

The thymus is persistent, well defined from the surrounding tissue and consists of two lobes, the right one of which extends in a finger-like projection up to the region of the thyroid. Its weight is 22 grams and its total length is 85 mm. Its color is pinkish, while its consistence and cut surface appearance is that of a thymus of a normal infant. Three small accessory thymi are found distributed among the cervical lymph-nodes, the highest point reached by any of them being

the lower pole of the thyroid; the largest of these accessory glands is 15 mm. in diameter, while that of the smallest is 5 mm.

The heart and its valves are normal in size and in appearance. The left ventricle is in firm systole. The aorta is elastic and its intima is smooth.

The liver weighs 2,100 grams; it is darker and softer than normal, and its cut surface bulges slightly from beneath the capsule.

The spleen is enlarged, weighing approximately 360 grams. It is a trifle soft. The cut surface is not abnormal in appearance except that the Malpighian bodies are inconspicuous.

The adrenals are of normal size and appearance. The left one weighs 5.5 grams. Both kidneys are considerably larger than normal. The left one weighs 161 grams. Their consistence is rather soft; the blood vessels are dilated. The cut surface is swollen; the cortex is mottled while the pyramids are pale.

The prostate, urinary bladder and ureters are normal. Unfortunately the testicles were not obtained. The external genitalia are well developed.

On opening the skull the calvarium is found to be very soft and thin. In places it is not over 1.5 mm. in thickness. The sutures of the skull are still distinct. There is a very little cerebrospinal fluid. The cerebral convolutions are broad and flat while the sulci are shallow and narrow. The brain is quite soft and dry; the lateral ventricles contain less fluid than normal. The sella turcica is enlarged so that it is 2 cm. deep and 2.5 cm. wide. It is tensely filled by the hypophyseal tumor, which has extended upward and backward into the third ventricle, distorting and partly destroying the infundibulum. Upward and forward it comes in contact with the posterior surface of the optic chiasma, which it has flattened out considerably. A fairly large flat tumor mass of rusty brown color and of a coarsely granular, spongy nature, extends anteriorly on the body of sphenoid bone, just posterior to the cribriform plate, which is intact. The bony wall of the sella turcica is thinned out from pressure of the tumor, but it is not invaded or broken through by the tumor mass.

Very conspicuous is the pineal gland which protrudes 5 mm. beyond the posterior edge of the corpus callosum. It is found to be 13 x 5 x 6 mm. in its various diameters, the antero-posterior diameter being the largest; its weight is 940 mg.

Microscopical Examination.—The alveoli of the thyroid gland are full of colloid, with an abundant number of desquamated parenchymatous cells.

The thymus gland resembles in all respects that of an infant; the capsule is thin and the lymphoid tissue more abundant at the periphery of the lobules than at the centers where the large lymphoid cells predominate. Hassall's corpuscles are numerous and large, their centers are often cyst-like and full of fatty cellular débris. The arterial walls are not thickened as in a thymus of a normal individual of this age, nor is there any fatty infiltration or perilobular fibrosis indicative of involution. The two accessory thymus nodules are of a similar structure.

All the lymph-nodes examined, including the mesenteric, the peribronchial and the cervical, represent a stage of active proliferation of the endothelial cells in the germinal centers as well as in the stroma. These endothelial cells are so numerous that the glands take a general pink stain with eosin.

The chromaffin cells of the adrenal have increased in size and in number, extending in some places through the cortical cell layer almost to the capsule.

In the spleen a condition of endothelial cell hyperplasia, similar to the condition in the lymph-nodes, is found.

Both the liver and the kidney present a slight connective tissue hyperplasia, and to some extent a swelling and granular condition of the parenchyma.

The heart muscle is of normal appearance.

The parathyroid glands are slightly larger than normal. The nuclei here, as in the adrenal medulla, take a deep stain with hematoxylin, indicating an abundance of chromatin material. A great many of the parenchymatous cells

here are vacuolated, while the cytoplasm of other remaining cells is solid, homogeneous, and deep pink staining with eosin. Colloid material is very scant.

The pineal gland is oval, being 13 mm. long and 6 mm. in depth. About the entire periphery of a sagittal section through the center of the gland there is a border of deeper staining tissue which is composed of cells with a diffuse, poorly outlined, and light pink staining cytoplasm, with large oval or pear-shaped vesicular nuclei; these nuclei average 13 microns in diameter but often reach 20 microns. The nucleolus is not prominent, at least not definitely distinguishable from small round masses of chromatin. Connective tissue trabeculae traverse this cellular border, extending from the thin capsule radially towards the center of the gland, dividing the cellular periphery into numerous compartments. Capillaries are fairly numerous and course mainly in the connective tissue trabeculae. The arrangement of the cellular border is, on the whole, in irregular cords and masses, and in ill defined alveolar structure, simulating tubules with an indefinite lumen of small caliber. At the tail end of the body, the parenchymatous layer is 3 mm. thick; the connective tissue trabeculae in this region are occasionally 30 microns thick and are quite vascular. No pigment (melanin) or any granules of a secretory nature can be seen. Psammomata are lodged in small groups, especially at the inner edge of the cellular border; they are not numerous. The evaginated pineal recess penetrates one-third the length of the gland; it is duct-like and is lined with low cuboidal epithelium. Intermixed with the parenchymatous cells of the border, are the small round and deep staining glial cell nuclei.

The carotid gland is of normal size and consists of small masses of chromaffin cells surrounded by thick connective tissue whorls.

The hypophyseal tumor is composed of masses of oval or round cells, with a fairly well defined cytoplasmic border. The nuclei are either oval or round and are deeply stained with hematoxylin; they average 7 microns in diameter. There is no set arrangement of these tumor cells although they assume an indefinite alveolar structure in places. Mitotic figures are extremely infrequent. In many instances two nuclei are found in one cell. With Van Gieson's stain there are seen delicate strands of connective tissue here and there in which run capillaries; compared to the normal hypophysis, the blood-supply and connective tissue are very scant. The tumor cells lie directly on the endothelium of the blood vessels as they do in sarcoma. The tumor mass is the seat of numerous diffuse hemorrhages and also regions of necrosis ranging from 30 microns to 2 mm. in diameter. The origin and nature of the cells composing this tumor are brought out clearly with granule stains, especially with acid-fuchsin methylene green. With this stain the cytoplasm of most of the cells is found to be filled with eosinophilic granules, corresponding to the eosinophilic type of chromophil cell of the normal pars anterior of the hypophysis. The granules in cells undergoing degeneration do not stain.

A macroscopic and microscopic examination of the tumor by Dr. D. D. Lewis revealed no traces of the posterior lobe; serial sections, however, were not made.

DISCUSSION

The polyglandular syndrome is represented here by (1) a chromophil cell adenoma of the anterior lobe of the hypophysis, (2) a persistent and non-involuted thymus, (3) a colloid goiter with marked desquamation of the parenchymatous cells, (4) hypertrophy of the chromaffine cells of the adrenal medulla, (5) hyperplasia of the endothelial elements of the lymph-nodes and of the spleen, (6) enlargement of the pineal body, and (7) atrophy of the cells of Sertoli and of Leydig (assumed on the basis of the loss of sexual function, and from other similar case reports).

In this case the hypophyseal disturbance is the probable underlying cause of the anatomical and functional upsetting of the other internal secreting glands, and without doubt it is the cause of the acromegalic symptoms. In the present state of our knowledge concerning the real cause of the hypophyseal growth, the possibility of fundamental biochemical disturbance affecting all of the glands at once, is not to be denied. Noticeable pressure symptoms are often lacking in the early hyperplasia of the hypophysis, even though acromegalic changes have occurred; this is explained by the fact that a hyperplasia of the chromophil cells may occur without appreciable concomitant enlargement of the gland. Some cases of acromegaly terminate fatally soon after the appearance of the acral changes, while other cases continue over a period of twenty years without causing any pressure symptoms. In other words, chromophil cell hyperplasia may be very insidious in its onset, and without gland enlargement extended enough to cause focal symptoms, but at the same time causing gigantism or acromegaly. On the other hand, hyperplasia of the anterior lobe may come on suddenly with rapid growth of the chromophil struma, causing death before there are any symptoms manifest of disturbed gland function.

In the case reported here, although clinical symptoms did not appear until a year before the death of the patient, hypertrophy of the anterior lobe, at least of the chromophil cells, would seem to have been present for ten years or more, because the thymus shows no signs of even a beginning involution; normally the thymus undergoes involution at about the thirteenth year.

The hyperplasia of the lymphoid structures of the intestines and lymph-nodes as well as of the spleen, is indicative of Paultow's status thymo-lymphaticus. Cushing cites two such cases; Claude and Boudouin[17] cite another case. It is not probable that the lymphatic enlargement here is part of a general tissue hyperplasia, since the increase of the lymphoid structures is out of proportion to the enlargement of the other tissues.

It is not known precisely what hypophyseal disturbance is responsible for a persistent thymus, and why in some cases it is present while in other apparently similar cases, it is absent. In dogs that live some time after hypophysectomy, the thymus usually remains persistent or enlarged. In some, but not all, of the cases of long standing hypopituitarism there is a persistent thymus; on the other hand, in several instances of hyperpituitarism of the anterior lobe, such as in Cushing's Case 38, in the case of Claude and Boudouin, and in this case, a persistent thymus is also present. By hyperpituitarism of the anterior lobe is understood in these

17. Claude, H., and Boudouin, A.: Etude histologique des glandes a secretion interne dans un cas d'acromegalie, Comp. rend Soc. de biol., Paris, 1911, lxxi, 75.

cases, a hyperplasia of the chromophil cells. In Graves disease the thymus is persistent and enlarged, according to Matti,[4] and to Capelle,[18] in 74 and 79 per cent. of the cases respectively; this would show that a hyposecretion of the anterior lobe of the hypophysis may be associated with a persistent thymus, since, according to Benda and Lewis, there is a decrease in the number of chromophil cells of the anterior lobe in cases of exophthalmic goiter.

Considerable variance of opinion exists concerning the relationship of the thyroid to the hypophysis. A condition of excess of colloid and of abundant parenchymatous desquamation, as in this case, has been found in all of Cushing's fifteen cases of dyspituitarism, where the thyroid was examined. It is his opinion that the thyroid is not functionally interchangeable with the hypophysis, but that both are simultaneously stimulated to hyperplasia by the same underlying biochemical disturbance.

In the case of giant Wilkins,[19] the thyroid gland weighed 112 grams and was of a colloid nature. In a chemical analysis of this thyroid Dr. H. G. Wells found that the total amount of iodin was 62.9 mg., or 2.28 mg. for each gram of the dried gland. In comparison to the iodin content of simple goiters from people living about Chicago and also from people living in the eastern states, Wells found that there is no difference in so far as the iodin content per gram weight of the dried gland is concerned. In simple goiters he found 1.5 to 2.5 mg. of iodin per gram of the dried gland.

Simpson and Hunter,[20] using the iodin content of the thyroid gland as the index to its activity, find no compensatory assumption of iodin by the hypophysis of sheep after thyroidectomy. Schonemann[21] examined the hypophyses and thyroids from 110 autopsies, including eighty-three cases of goiter. The results which are indicative of some relation between the glands are as follows:

27 cases of normal thyroid	Hypophyses all normal.
30 cases of colloid thyroid	Increase of the chromophil cells.
1 case of normal thyroid	Increase of the chromophil cells.
33 cases of colloid thyroid	Increase of the connective tissue.
10 cases of colloid thyroid	Increase of the vascularity.
9 cases of colloid thyroid	Increase of the colloid content.

Caselli[11] and Cushing both noticed hyperplasia of the thyroid gland after hypophysectomies; later, in the chronic stage, the thyroid became colloidal.

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18. Capelle: Cited by Charles A. Parker. Note 3.

19. Bassoe, P.: Gigantism and Leontiasis Ossea, Trans. Chicago Path. Soc., 1901-1903, v, 231.

20. Simpson and Hunter: Does the Pituitary Body Compensate for Thyroid Insufficiency, Proc. Soc. Exper. Biol. and Med., 1910, viii, 5.

21. Schonemann, von A.: Hypophysis und Thyroidea, Virchows Arch. f. path. Anat., 1892, cxxix, 310.

In one case reported in the literature by Claude and Boudouin, the parathyroid glands were described as being four times as large as normal; no case of gigantism or of acromegaly could be found recorded in the literature in which parathyroid insufficiency, producing tetany, was evident.

Cushing has pointed out that in several of his cases, symptoms of hypoadrenalism were present, i. e., low blood-pressure, pigmentation of the skin, and hypoglycemia. In two other cases there were symptoms of hyperadrenalism, manifested by a high blood-pressure. In one of these latter cases, the adrenal medulla was the seat of a hyperplasia of the chromaffin cells; both adrenals together weighed 15.7 grams. In many other cases reported in the literature, hyperplasia of the medulla and vacuolization of the zona fasiculata are conspicuous.

As exemplified by this case, the interrelation of the hypophysis to the testicles and ovaries is a very close one. In hypophysectomized puppies, the testicles remain small and undeveloped, while in old dogs, hypophysectomy leads to impotence and atrophy of the testicles.[22, 1] Practically no secondary testicular change has followed experimental hyperpituitarism. Physiological hypertrophy of the hypophysis occurs during puberty, pregnancy and the climateric, indicating a close relation between the hypophysis and the sex organs.

Clinically, hypopituitarism, almost constantly associated with hypophysical tumors, leads to undeveloped ovaries and testicles, and to infantile sex characteristics, when occurring in young people; in adults, anaphrodisia, impotence, amenorrhea, and reversive sex characteristics result. It is probable that hyperpituitarism, such as occurs in early chromophil cell hyperplasias primary to acromegaly, leads to a transient sexual excitability. In Case 1 of Cushing's series, the history reveals an early excessive libido, while later there was impotence, after pressure symptoms and acromegaly had intervened. In my case, abnormal libido was the first sexual symptom noticeable.

The pineal gland in this case is of special interest, since it is over twice as large as normal. I have been unable to find any reference to its simple enlargement in connection with hypophyseal or with polyglandular disturbance. The physiological action of the pineal gland, if at all of importance, must be so during the latter part of intrauterine life and after birth, until the tenth or fifteenth year. At birth in man, it is the size of a grain of wheat; at the age of one year it is 3 mm. long, while at puberty it averages 7x5x4 mm., the largest size of all periods. From puberty on it becomes smaller, fibrous, cystic and filled with psammomata. Ontogenetically considered, it is the remains of the third eye or pair of

22. Aschner, B.: Demonstration von Hunden nach extirpation der Hypophyse, Wien. klin. Wchnschr., 1909, xxii, 1730.

eyes, found now in one species of Australian lizard, the *Hatteria punctata*.[23] In Jordan's[24] excellent work on the development of the pineal body in sheep, where it is much larger than in other common mammals, he finds that in the 1.5-months-old embryo, there develop in the gland unbranched, blind alveoli, 80 microns long. These alveoli are composed of low columnar cells with an indistinct cytoplasmic outline; melanin granules are almost constantly found in the cytoplasm of these cells. At one half term (2.5 months), tufts of capillaries running in the connective tissue septa are invaginated into these tubules, forming structures not unlike the Malpighian bodies of the kidney. At term (5 months), the gland reaches its greatest development, being then 8x5x5 mm. in size, and containing numerous alveoli with many glomerular invaginations. Jordan could find no evidence of secretory activity other than the melanin granules. At 8 months after birth, the time of mating in sheep, the pineal body is still as large as at birth. After the first year it decreases in size, finally becoming small, sclerotic and cystic in old sheep. He concludes that its sphere of development up until puberty, together with its gland-like structure, makes it appear very much like a gland of internal secretion; on the other hand, all these phenomena of development can be interpreted on a phylogenetic basis.

Krabbe,[25] in the study of 100 human pineal glands, found what he interpreted as being secretory granules of a basophilic nature. The granules appear in the nuclei and are shed into the surrounding cytoplasm; this process occurs constantly up until advanced age. Kidd[26] believes that the pineal gland ceases in activity as the hypophysis waxes in function, having reference here perhaps to the fact that the chromophil cells of the hypophysis increase from birth on until 40.

Exner and Boese[27] extirpated the pineal gland in ninety-five young dogs, and observed several until puberty without noticing any physiological changes in their development. The injection experiments of von Cyon,[28] Howell,[29] Dixon and Halliburton[30] and others, with extracts

23. Bailey and Jelliffe: Tumors of the Pineal Body, THE ARCHIVES INT. MED., 1911, viii, 851.

24. Jordan, H. E.: The Histogenesis of the Pineal Body in Sheep, Am. Jour. Anat., 1911, xii, 249.

25. Krabbe: Sur le gland pineal chez l'homme, Nouv. iconog. de la Saltpétrière, 1911, p. 257.

26. Kidd, L. J.: Pineal Experimentation, Brit. Med. Jour., 1910, ii, 2202.

27. Exner, A., and Boese, J.: Ueber experimentalle Extirpation der Glandula-pinealis, Deutsch. Ztschr. f. Chir., 1910, cvii, 182.

28. Von Cyon, V.: Zur Physiologie der Hypophyse, Arch. f. d. ges. Physico., 1901, lxxxvii, 389.

29. Howell, C. M. H.: Tumors of the Pineal Body, Royal Soc. Med., London, March, 1910.

30. Dixon, W. E., and Halliburton, W. D.: The Pineal Body, Quart. Jour. of Exper. Physiol., 1909, ii, 283.

of the pineal body, were all practically negative. Dixon and Halliburton used extracts of adult sheep glands; as all evidence points to the fact that the function of the pineal gland is one of early life, it would be expected that extracts of adult pineal glands were inert.

Jordan and Eyster,[31] using fresh extracts of sheep pineal glands and injecting these into cats, dogs and sheep, obtained a slight fall of blood-pressure, greater than with extracts from other parts of the brain. Perfusion of two cats' hearts, using one gland to a liter of salt solution, produced a slight strengthening and quickening of the beat. In most dogs a short period of diuresis was produced by injection of acidulated extract. The author does not state whether or not he used glands from lambs or from sheep.

Foa[32] found in three young roosters from which the gland had been removed before the fifth week, that the testes and combs, at the eleventh month, were larger than those of the controls. These depinealizd roosters crowed thirty-six days earlier, mated forty-seven days sooner, and were a little heavier than the controls. There were no macroscopic changes in the other glands. Biach and Hulles[33] removed the reproductive organs from kittens; seven months later they found that there was atrophy not only of the whole pineal gland, but also of its individual parenchymatous cells, as compared to the glands of normal kittens from the same litter.

Bailey and Jelliffe collected from the literature up until 1912, fifty-nine cases of pineal tumor, including adenomas, sarcomas, adenosarcomas, teratomas, glomas, endotheliomas and carcinomas. Four of these fifty-nine cases, namely, those of Ogle,[34] a sarcoma; Oestreich and Slawyk,[35] a psammomata cysticus; Marburg,[36] a teratoma, and Frankel-Hochwart,[37] a teratoma, all under 10 years of age, had developed the remarkable sydrome of precocious sexual maturity. For example, in Frankel-Hochwart's case, the boy at the age of 3 began to grow rapidly, so that at 5 he had reached the size of a boy of 7 years; his mind dwelt on adult subjects; his penis was large, while pubic and axillary hair were present; erections

31. Jordan, H. E., and Eyster, J. H. E.: The Physiological Action of Extracts of the Pineal Body, Am. Jour. Physiol., 1911, xxix, 115.

32. Foa, C.: Hypertrophy of Cocks' Combs and Testes Following Removal of the Pineal Gland, Arch. ital. de biol., Turin, 1912-1913, lvii, 233.

33. Biach, P., and Hulles, E.: Ueber die Beziehungen der Zirbeldruse zum Genitale, Wien. klin. Wchnschr., 1912, xxv, 373.

34. Olge, C.: Sarcoma of the Pineal Body, Trans. Path. Soc. London, 1899, l.

35. Oestreich and Slawyk: Risenwuchs und Zirbeldrusen geschwulst, Virchows Arch. f. path. Anat., 1899, clvii, 475.

36. Marburg, O.: Zur Kenntnis der normalen und pathologischen Histologie der Zirbeldruse; Die Adipositas Cerebralis., Deutsch. Ztschr. f. Nervenh., 1909, xxxiv, 114.

37. Frankel-Hochwart, von L.: Ueber Diagnose der Zirbeldrustumoren, Deutsch. Ztschr. f. Nervenh., 1909, xxxvii, 455.

were frequent; his voice had changed. At 9 years he was as large as a boy of 15, except that his genitals were those of a boy of 17 or 18.

A case reported by Cushing of a boy 8 years old with similar symptoms of precocious sexual development, may be included in this class of pineal cases, although exploratory operation revealed nothing obviously abnormal with the pineal body.

Marburg was the first to lay emphasis on this syndrome. The metabolic symptoms, according to him, are (1) precocious sexual development due to hypopinealism, (2) universal adiposity due to hyperpinealism, and (3) cachexia due to apinealism. Adiposity has been found in about ten cases of pineal tumor unassociated with sexual disturbance; this adiposity may be very well explained, aside from whatever specific effect the pineal gland may have in governing carbohydrate metabolism, by a hyposecretion of the posterior lobe of the hypophysis, resulting in turn from a third ventricle hydrocephalus; occlusion of the duct of Sylvius by tumors located in the pineal region frequently results in hydrocephalus of the third ventricle, a condition often associated with adiposity.

Whether the enlargement of the pineal body, in the case reported here, is due to some primary biochemical disturbance, or is secondary to the hypophyseal disturbance, cannot be determined. If the pineal gland is one of the glands of internal secretion, and in all probability it is during early life, its abnormal enlargement here, as but one manifestation of a polyglandular syndrome, is of considerable significance.

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THE FUNCTIONS OF THE DIAPHRAGM AND THEIR DIAGNOSTIC SIGNIFICANCE*

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A clear understanding of the respiratory rôle of the diaphragm is necessary to analyze the respiratory movements in the thorax and abdomen. In former years the inspiratory excursion of the diaphragm was grossly exaggerated by estimating the excursion of the lower borders of the lungs as a measure of phrenic movement. These were misleading because the descent of the lung in the pleural sinus to an extent of 6 centimeters may be accomplished by the diaphragm descending or withdrawing from the thoracic wall only a distance of 1 centimeter.

The inspiratory descent of the anterior hepatic border is an exaggeration of phrenic movement because the liver rotates on a transverse axis during inspiration and is not depressed in toto during inspiration. This axis of rotation is marked by the posterior hepatic border or that liver portion which is not covered by peritoneum. This part of the liver is fixed by reflections of the peritoneum from the liver to the under surface of the diaphragm and posterior abdominal wall from the superior and inferior surfaces, respectively, of the liver.

Inspiratory movements of the diaphragm, therefore, rotate the liver forward and downward. The rotating force is applied on the posterior and superior portion of the upper surface of the liver and therefore the excursion of the anterior border of the liver corresponds to the excursion of a lever of the third class, the fulcrum being at the posterior hepatic border and the power applied at the posterior portion of the upper surface of the liver. So the descent of the lower pulmonary and anterior hepatic borders are gross exaggerations of phrenic excursion.

In recent times the fluoroscope has given us more accurate conceptions of the inspiratory descent of the diaphragm. A very good example of this magnification of phrenic descent is seen in pericarditis with effusion. Several years ago a patient with purulent pericarditis of pneumococcus origin came under my observation. The liver in this instance presented a great rounded tumor on the right side of the abdomen with its anterior border rotated downward in the iliac fossa. Paracentesis of the pericardial sack was performed, and although only 700 c.c. of pus were

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removed, when the aspiration was finished, the anterior border or edge of the liver occupied its normal position at the right costal border.

All discussions of the function of the diaphragm which have appeared in recent years contain recognition of two movements at the base of the thorax which are caused by diaphragmatic contractions: (1) retraction of the hypochondria, and (2) widening of the subcostal angle or divergence of the costal borders.

Keith,[1] in his article on "Respiration in Man," refers to Duchenne's experiments and deductions which amounted to proving that diaphragmatic action contributed to widening of the subcostal angle and spreading of the hypochondria by virtue of the piston action of the liver, spleen and stomach, which are driven downward and forward, thus spreading the upper lateral abdominal boundaries. All modern works in physiology and anatomy refer to Duchenne's experiments and unite in maintaining the validity of his deductions.

When one attempts routinely to analyze the respiratory excursion of the thoracic walls in a service of patients with varied thoracic and abdominal conformations and with varied thoracic and abdominal diseases, it will be found that Duchenne's explanation is not tenable.

1. It is proposed to show in the following discussion that the resultant of phrenic contraction always tends to narrowing of the subcostal angle, retraction of the lower end of the sternum and retraction of the internal portions of the hypochondria above the level of the ninth costal cartilages.

2. Although the action of the diaphragm is correlated with the action of the scaleni and intercostal muscles, the diaphragm is the antagonist of the other muscles in their efforts to enlarge the transverse and anteroposterior diameters of the base of the thorax, and also acts as an antagonist to the tendency of these muscles in their inspiratory activity to lessen the longitudinal diameter of the thorax and lengthen the longitudinal diameter of the abdomen.

3. There is a two-fold purpose in the contraction of the diaphragm, viz., to maintain the integrity of the longitudinal diameter of the thorax against the inspiratory effect of the scaleni and intercostals, and also to enlarge the longitudinal diameter of the thorax.

4. The resulting variations in respiratory movements of the lower end of the sternum, subcostal angle and costal borders from the ninth rib to the subcostal angle, the epigastrium and hypochondria, all depend on the higher or lower positions of the diaphragm.

5. When the diaphragm is pushed upward it is placed at a mechanical disadvantage in approximating the points of origin and insertion of its muscle, viz., the central tendon, sternum and costal borders.

1. Keith: Further Advances in Physiology, 1909, p. 182.

But when the diaphragm is displaced downward the muscle is placed at a mechanical advantage for its effect on the movement of these structures.

6. However high or low the diaphragm may be placed, so long as its muscle is active the longitudinal diameter of the thorax is either maintained or enlarged by its action.

Although all these propositions seem very simple and are easily explained, the want of a satisfactory understanding of the function of the diaphragm has made it impossible to make a critical analysis of respiratory movements of the thorax in any and all diseases.

The so-called Litten diaphragm phenomenon is of service in estimating the excursion of the lower border of the lungs, but it is such a small part of the many evidences of respiratory movement at the lower thoracic aperture, that when it is absent the phenomenon is not of any diagnostic service. Several monographs[2] on the diaphragmatic function have been published from German sources, but they deal only with the effect of phrenic contraction on the pleural sinus and treat the excursion of the costal angle evasively. The more important manifestations of diaphragmatic activity are disregarded because the antagonistic relations between the diaphragm and scaleni have not been taken into account.

In the dog in health and disease the diaphragm and its antagonists produce respiratory movements of the lower end of the sternum, subcostal angle, costal borders, epigastrium and hypochondria, identical with those seen in human beings. In man, spasm of the diaphragm causes retraction of the lower end of the sternum, narrowing of the subcostal angle and violent approximation of the hypochondria toward the median line. When this function is seen in diaphragmatic pleurisy or mediastinitis in man we are in doubt about the movements resulting from diaphragmatic action alone. The transverse abdominal muscles may also contribute to the movements. If the linea alba of the dog is slit from the sternum to the pubes and the abdominal walls on both sides are cut transversely to the quadratus lumborum then muscular activity of the abdominal walls can be eliminated as a factor in movements of the costal borders.

After these preparatory incisions, electrodes are placed on the right and left leaflets of the diaphragm. When the diaphragm is then stimulated by a strong electric current the sternum, subcostal angle, costal borders and hypochondria exhibit the same movements as are seen during phrenic spasm in man. When the three roots of the phrenic nerve of the dog are cut on both sides so the diaphragm is completely paralyzed, then the inspiratory widening of the subcostal angle and flaring of the hypo-

2. Zabel, Erich: Das Spiel des Zwerchfells, Berlin, 1906, p. 176; Eppinger: Allgemeine und Spezielle Pathologie des Zwerchfells, Wien, 1911, p. 252.

chondria during inspiration are much exaggerated over the same movements which occur when the phrenic nerves are intact.

A few months ago we had a child at Lakeside Hospital whose arms and legs and diaphragm were completely paralyzed from poliomyelitis anterior. The scaleni and intercostal muscles were intact. The respiratory movements were identical with those of the dog whose phrenic nerves were severed.

The subcostal angle showed an exaggerated widening during inspiration. The costal borders and hypochondria diverged from the median line, and the epigastrium was retracted during inspiration. In other words, the scaleni and intercostals had no antagonist to modify their inspiratory movements of the hypochondria and costal borders.

A man, 47 years old, was operated at Lakeside Hospital six months before his second admission to the hospital on account of empyema of the gall-bladder; drainage was established and in about two months the drainage from the gall-bladder ceased, the sinus closed and the patient was perfectly comfortable during a period of four months, when suddenly he was seized with chills and fever and severe pain in the right hypochondrium and over the right deltoid muscle. A week later he was admitted to the medical wards and presented all the characteristic physical signs of a large amount of fluid in the right pleural cavity. The right thorax was larger than the left. The right side was flat on percussion from the clavicle to the edge of the liver which was located 2 inches below the costal border in the nipple line. The physical signs of auscultation and percussion were characteristic of a pleural cavity filled with fluid. The one sign which was at variance with thoracic empyema was the fact that the affected side had a larger excursion, and particularly the right costal border diverged much more from the median line than did the left costal border. This fact indicated a loss of function of the diaphragm and suggested that an infraphrenic abscess had ruptured through the diaphragm into the right pleural cavity, or at least a myositis of the diaphragm had resulted in a loss of its muscular function. For, had the diaphragm been displaced downward by the empyema and had the phrenic musculature been left intact then the activation of the phrenic muscle would have fixed the right costal border so as to prevent its divergence from the median line; or the right costal border would have been drawn toward the median line during inspiration. Evidently the scaleni and intercostal muscles of the right side were not opposed by the normal antagonistic action of the diaphragm. Unfortunately, the patient's critical condition at the time of operation did not admit of definitely determining whether there was or was not an opening through the diaphragm. Two weeks after the empyema was drained the patient's costal borders moved symmetrically during inspiration.

Another patient developed signs suggesting thoracic empyema following appendicitis. The base of the right thorax was dull, tactile fremitus was diminished. Auscultation revealed a diminution of the intensity of the respiratory sounds attended with elevation in pitch of the inspiratory and expiratory sounds.

All these physical signs were strongly suggestive of thoracic empyema, but one sign alone gave the key to the diagnosis. The costal border of the affected side had a greater lateral excursion than the sound side. This fact could have only one interpretation, and that was a loss of the normal antagonism of the diaphragm to the action of the scaleni and intercostals of the affected side.

In conjunction with the other physical signs mentioned, this sign indicated an upward displacement of the diaphragm and led to the diagnosis of a subphrenic abscess.

An operation was performed to establish drainage and the entire accumulation of pus was found beneath the diaphragm.[3]

Upward displacement of the diaphragm diminishes the inspiratory restraint of the costal borders because the more its muscle is curved the less will be the resulting traction on its sterno-costal attachments. Conversely, the flatter the diaphragm the greater will be the resulting traction on its sterno-costal attachments.

When hepatic enlargements displace the diaphragm in an upward direction, then the right costal border will exceed the left costal border in its inspiratory divergence from the median line. Patients with acute jaundice who have swollen livers show this disparity in movement. After the jaundice has subsided and the diaphragm resumes the original position then the two costal borders are seen to have a symmetrical excursion.

When the liver is enlarged in an upward direction sufficiently to displace the lower border of the lung a distance of one intercostal space this increase in the excursion of the costal border is distinctly visible. Paresis of the diaphragm or accentuation of the convexity of the diaphragm will serve to increase the inspiratory divergence of the costal border from the median line. An upward displacement of the diaphragm will not serve to increase the movement of the costal border on the affected side if the displacement is caused by an obsolete pleurisy with synechia between the diaphragm and the thoracic wall. This is particularly true when the site of the synechia is in the anterior portion of the pleural sinus.

A patient who recovered from bilateral pleurisy had the usual characteristic signs of obsolete pleurisy, viz., dulness, diminished excursion,

3. This observation was made by Dr. H. L. Taylor, resident physician at Lakeside Hospital.

diminished tactile fremitus and lessened intensity of the respiratory murmur without elevation in pitch of the respiratory sounds or any adventitious sounds. The right costal margin moved away from the median line during inspiration, but the left costal border moved toward the median line during inspiration. The physician who attended the patient during his illness said the most persistent and most intense dulness and resistance was over the left inframammary region where the most marked signs of thickened pleura and synechia persisted.

This evidence of increased diaphragmatic retraction of the costal border suggested that the diaphragm had acquired a mechanical advantage in its traction on the costal border because there was fixation of the diaphragm to the thoracic wall above the costal border, thus giving approximately a straight line to the phrenic muscular strands from the central tendon to the left sterno-costal region.

This view was confirmed by the following very simple experiments:

The abdomen of a dog was opened, and to avoid the possibility of admitting air to the pleural cavity a thin strip of wood was placed against the under right side of the diaphragm, so that the diaphragm over the site of the pleural sinus from the right sternal border to the anterior axillary line was brought into contact with the thoracic wall by a clamp which was applied to the outer side of the thorax and the inner surface of the strip of wood. This gave a high insertion of the diaphragm and resulted in fixation and retraction of the right costal border during inspiration. The same effect on the inspiratory excursion of the costal border was procured by fixing the diaphragm to the ribs about 2 inches above the costal margin by means of silk ligatures which were passed through the diaphragm and thoracic wall with very thin needles. When the ligatures were released the costal border resumed its original inspiratory divergence from the median line.

This manner of upward displacement of the diaphragm converts the line of traction from a curved to a straight line, thus placing the diaphragm at a mechanical advantage, whereas an upward displacement of the diaphragm which accentuates the curve of the line of traction (as occurs in hepatic enlargement) will place the diaphragm at a mechanical disadvantage. The movements of the costal borders and lower end of the sternum are much more readily affected of course by varying elevations and depressions of the anterolateral strands of the diaphragm than the changes in the elevation of the posterior strands of the diaphragm. This is apparent from clinical experiences and also from trials made in the experiment on the dog which I have just described. When the posterior portion of the diaphragm was fixed to the thoracic wall over the site of the pleural sinus, the inspiratory widening of the subcostal angle was not modified.

Thus far the discussion has dealt with clinical and experimental evidences which show the subcostal angle is enlarged during inspiration by the action of the scaleni and intercostal muscles, and also that impairment of the diaphragmatic muscle or paresis from disease or cutting the phrenic nerves or accentuation of the arch of the diaphragm will contribute to the inspiratory widening of the subcostal angle. We can now see the errors involved in the interpretation of Duchenne's experiment of driving the diaphragm upward by bandaging the abdomen of the dog and thus increasing the longitudinal diameter of the abdomen at the expense of the longitudinal diameter of the thorax. The accepted interpretation of this experiment is, the diaphragm thus being prevented from encroaching on the longitudinal diameter of the abdomen by its contraction, expends its force in elevating the costal borders and widening the subcostal angle.

The correct interpretation of Duchenne's experiment lies in the fact that an increase of the convexity of the diaphragm without altering its insertion in the antero-lateral thoracic wall, places the diaphragm at a mechanical disadvantage and thus contributes to widening of the subcostal angle by the action of the scaleni and the intercostal muscles.

Downward displacement of the diaphragm will place the diaphragm at a mechanical advantage in restraining the divergence of the costal borders, and thus causes a diminution of the inspiratory widening of the subcostal angle, and may cause retraction of the lower end of the sternum, narrowing of the subcostal angle and approximation of the hypochondria toward the median line.

My attention was first called to these phenomena in pulmonary emphysema. A moderate degree of pulmonary emphysema will cause the pleural sinuses to be filled with the enlarged pulmonary borders and still the emphysema will not be severe enough to cause cyanosis or an increase of the CO_2 content of the alveolar air above 5.3 per cent. Under these conditions there will still be a distinct widening of the subcostal angle during inspiration. If the degree of emphysema increases, of course the lower borders of the lung can descend no further than the bottom of the pleural sinus, but farther flattening of the diaphragm causes the subcostal angle to be narrowed during inspiration and the anterior hypochondria will be approximated toward the median line during inspiration. When emphysema is sufficiently severe to cause cyanosis, so that the alveolar air will contain 6.5 per cent. or more of CO_2, then the subcostal angle will not be enlarged or it will be diminished during inspiration.

Variations of degree in pulmonary emphysema offer very good opportunities to see just when the critical point in flattening of the diaphragm is obtained.

The first step in emphysema reveals the pleural sinus filled with lung; then as the flattening of the diaphragm progresses, the critical point is attained when the subcostal angle remains constant during inspiration, and further descent of the diaphragm is attended with inspiratory narrowing of the subcostal angle and inspiratory approximation of the hypochondria. The last condition is always seen when the alveolar air obtains a percentage of CO_2 as high as 7.5 per cent.

When pulmonary emphysema is attended with cyanosis and these changes in the respiratory movement of the subcostal angle and hypochondria are not apparent, then it can be said with certainty that the cyanosis is not due to impairment of lung ventilation from emphysema alone, but the cyanosis must then have a contributory cause in some other factor which impairs either internal respiration or the respiratory function of the lung, which is quite distinct from the ventilatory function. Conversely, when an emphysematous patient has marked inspiratory narrowing of the subcostal angle and cyanosis is absent, then some other factor besides enlargement of the lung must contribute to the flattening of the diaphragm. Enlargement of the pericardial sac causes inspiratory narrowing of the subcostal angle.

A small pericardial effusion may be demonstrated by other physical signs when the phrenic displacement is so slight that it allows inspiratory widening of the subcostal angle. As the effusion increases the critical point is reached when the subcostal angle remains constant during all respiratory phases and the epigastrium is protruded during inspiration. When accumulation of fluid in the pericardial sac progresses there will be inspiratory narrowing of the subcostal angle and inspiratory retraction of the epigastrium.

It is this stage of diaphragmatic depression which has often been interpreted as phrenic palsy in pericarditis. The diaphragm is not paralyzed, but it is activated during inspiration, and because of its downward displacement retracts the hypochondria and narrows the subcostal angle. Owing to the flattening of the diaphragm, its activation does not cause a further lengthening of the thorax, but the longitudinal diameter of the thorax is maintained and the epigastrium is retracted because the thoracic cage in toto is elevated. This elevation of the thoracic cage enlarges the capacity of the hypochondria; consequently the epigastrium is retracted.

Within a day's time the pericardial effusion may be absorbed sufficiently to cause a return to the normal type of respiratory movement of the subcostal angle and the epigastrium. This lack of respiratory excursion of the diaphragm attended with inspiratory retraction of the epigastrium has been incorrectly described as paralysis of the diaphragm in pericarditis. There can be no paralysis of the diaphragm when the

subcostal angle is narrowed during inspiration. Paralysis of the diaphragm contributes to the inspiratory widening of the subcostal angle. We have had ample opportunity to confirm at Lakeside Hospital this effect of pericardial effusion and the inspiratory movement of the subcostal angle and costal borders. A patient with pericarditis who was frequently tapped always exhibited the phenomena above described before and after each paracentesis.

These phenomena are specifically described because one finds in all neurological works very unsatisfactory discussions of the symptoms of paralysis of the diaphragm. And where specific statements are made, as in La Pratique Neurologique by Guillain,[4] we find the symptoms of phrenic paralysis are described as showing "inspiratory retraction of the hypochondria and expiratory expansion of the hypochondria which is the opposite of what is normally seen." This error has evidently arisen from mistaking the evidences of a flattened diaphragm for evidences of diaphragmatic palsy.

Cardiac enlargement may alter the respiratory excursion of either the left or both costal margins. If the enlargement is to the left of the median line, only the left margin is affected; if the enlargement is to the median line, as occurs in mitral stenosis, then the subcostal angle will be symmetrically narrowed during inspiration.

Thus far only those conditions which cause a widespread flattening of the diaphragm (as in emphysema), or those which cause purely a local flattening of the sterno-costal fibers of the diaphragm have been described.

Filling of the pleural sinus laterally and posteriorly will also modify the inspiratory excursion of the costal border on the affected side. This effect is of great service in recognizing pleurisy with effusion and pneumothorax, as both conditions will result in fixation of the costal border or an inspiratory traction of the costal border toward the median line.

For example, a young girl 18 years old, had a pleurisy with effusion on the right side which was unattended with pain. The fluid occupied only the posterior aspect of the pleural cavity. During inspiration the left costal border diverged from the median line. The right costal border was drawn toward the median line during inspiration. Two hundred and seventy c.c. of fluid was aspirated and then the right costal border diverged from the median line.

Pleuritic pain accompanying an inspiratory movement will inhibit the excursion of the costal margin during inspiration, but this is quite different from restraint of the costal border in pleurisy with effusion. When pleuritic pain is a factor, the scaleni and intercostal muscles of the affected side are inhibited in their action, so of course the costal

4. Marie, P.: 1911, p. 650.

border will move very little, but the costal border is not drawn toward the median line. The important point to bear in mind is to determine the movement of the costal border when the actions of the scaleni and intercostals are not inhibited.

This movement of the costal border is frequently of great service in differentiating between pulmonary infiltration of the lower lobe of a lung and pleurisy with effusion, and also in differentiating between an obsolete pleurisy and pleurisy with effusion when the obsolete pleurisy does not involve the sterno-costal portion of the diaphragm.

Consolidation of the lower lobe of the lung and obsolete pleurisy may diminish the excursion of the costal margin, but there will be some movement and that will be away from the median line, and not toward it, as occurs in pleurisy with effusion.

Pneumothorax causes inspiratory traction of the costal border toward the median line on the affected side, as can be readily seen in man and produced in dogs by the admission of air to a pleural cavity. Air was admitted to the right pleural cavity of a dog and directly the costal margin of the right side was seen to move toward the median line during inspiration.

All the roots of the right phrenic nerve were then cut and directly the right costal border diverged from the median line during inspiration, although the diaphragm occupied a very low position. The diaphragmatic attachments to the right costal border were then cut and the lateral excursion of the right costal border during inspiration was greatly increased.

Keith[1] says, "The writer, from observations made by the x-rays, has come to regard a free movement of the subcostal angle as an index of a free action of the diaphragm." This statement is literally true, but not in the sense in which Keith interprets the phenomenon. If the diaphragm is activated in a normally arched position or in a position higher than normal, there will be a marked excursion of the diaphragm and inspiratory widening of the subcostal angle. In this sense activation and excursion of the diaphragm are attended with widening of the subcostal angle, but the inspiratory widening of subcostal angle is not due to activation of the diaphragm, but to activation of the muscles which lift the thoracic cage. When the convexity of the diaphragm is flattened its activation will be quite as strong as when the diaphragm has a maximum convexity, but phrenic activation in a lowered position manifests itself in fixation or narrowing of the subcostal angle.

Briefly stated, movements of the costal borders during respiration give valuable aid in differentiating between supraphrenic and subphrenic disease. Also in differentiating between pulmonary consolidation and

pleurisy with effusion, and in differentiating between obsolete pleurisy and pleuritic effusion.

Costal border movements enable us to form a comparative estimate of a degree of pulmonary emphysema which exceeds mere filling of the pleural sinus with aerated lung. Movements of the costal borders give indication of the amount of fluid in a pericardial sack and also contribute to an appreciation of cardiac enlargement and an appreciation of the relative enlargements of the two ventricles. Unlike the diaphragm phenomenon of the pleural sinus, these movements of the costal borders and the subcostal angle are always present and can be accurately observed whatever the disease may be and however thick may be the panniculus adiposus. So these signs have a much broader and more accurate diagnostic significance than the so-called Litten diaphragm phenomenon.

In conclusion a word of caution should be added. In making an estimate as to whether a costal border is stationary or diverges from, or is drawn toward the median line, the examiner should apply his thumbs symmetrically placed along the costal borders to serve as indicators. Inspection alone without the aid of palpation of the borders may lead to confusion between elevation of the thoracic cage and widening of the subcostal angle. The thoracic cage may be strongly elevated and still the subcostal angle remain the same size. In fat persons, asymmetrical movements of the costal borders may be obscured without the aid of indicatory palpation.

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ON THE DEMONSTRATION OF VARIATIONS IN THE THYROID COLLOID IN CONDITIONS OF HYPER- AND HYPOTHYROIDISM *

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The experiments here reported were undertaken in an attempt to demonstrate functional variations of the thyroid. From an analogy with other glands of the body, such variations probably exist in different individuals, and also in the same individual under different conditions. Heretofore there has been no method sufficiently dependable to follow even the extremes of activity of the gland, not to speak of the many possible intermediate stages. Until a satisfactory method is developed, no definite knowledge is obtainable of the true character of the various conditions supposedly referable to abnormal states of the thyroid gland.

One of us[1] has for a considerable time been interested in the development of a biological test for the thyroid secretion. Attempts are being made to use the cretin rabbit for this purpose, yet by the technic so far employed satisfactory results have not been obtained.

The Hunt acetonitril test, though valuable for certain lines of attack, has failed in the hands of Carlson and Woelfel,[2] Hunt[3] and Lussky[4] to determine any constituent of the thyroid product in the blood of dogs, rabbits or guinea-pigs in any experimental condition of hyperthyroidism. With the blood of clinical cases of exophthalmic goiter, Hunt obtained positive results in two out of three cases. Carlson and Woelfel could not get a positive reaction in the case studied by them, nor in Carlson's own blood after having taken thyroid till disagreeable symptoms arose.

Very recently one of us[5] has shown Mallory's connective tissue stain, when applied to the colloid in tissues fixed in Zenker's solution, to be capable of indicating variations in the iodin content. It was thought that the iodin variations might well be parallel to, or dependent on, a functional thyroid activity, and hence by such a method, variations in thyroid activity might well be followed. The work reported in this paper is based on the estimation of relative amounts of iodin in thyroid colloid as

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1. Tatum, A. L.: Jour. Exper. Med., 1913, xvii, No. 6.
2. Carlson, A. J., and Woelfel, A.: Am. Jour. Physiol., 1910, xxvi, 32.
3. Hunt, Reid: Jour. Am. Med. Assn., 1907, xlix, 240.
4. Lussky, H. O.: Am. Jour. Physiol. 1912, xxx, 63.
5. Jones, A. P.: Jour. Exper. Med., 1913, xvii, No. 5.

indicated by the reaction with the modified Mallory's stain. Although other molecular groups than those containing iodin may stain blue with the stain used, yet the rough parallelism of amount of blue staining colloid to the analyzed iodin content of the gland, together with increased blue colloid on iodid administration, indicate very strongly that the blue staining reaction is due to the iodin in the colloid. Obviously we are entirely in the dark as to the character of the iodin combination, either in the colloid of the gland or in the circulation; but even if subsequent work showed that this staining reaction is not due to iodin, the least that can be said is that the extent of this staining reaction runs parallel to the iodin concentration. We are compelled to follow the unknown molecule by the single element iodin, and that we determine only as it occurs in the thyroid colloid.

The manipulative procedures were the following: After aseptically removing a small piece of one thyroid lobe, rabbits were fed on commercial desiccated sheep thyroids[6] for three or four days until, with very moderate doses, distinct toxic signs were seen. Then another small piece was removed from the remaining intact lobe, after completion of the period of thyroid administration. The thyroid colloid showed in every case a marked increase in the blue staining reaction.

These thyroid-fed animals were bled, and serum was prepared by coagulation and centrifugalization. This serum was injected intravenously into a test rabbit from which a small piece of thyroid had previously been removed for control. Forty-eight hours later a portion of the remaining intact lobe was removed, fixed in Zenker's solution and stained by the method above mentioned.

This procedure results in an accumulation of iodin in the thyroid of the test animal, as indicated by the increased area of blue staining colloid. This result was constant in all but a single case (see table), in which the second portion of thyroid was removed after twenty-four instead of forty-eight hours. Several animals showed a positive reaction at twenty-four hours, but in view of the apparent exception, we thereafter removed the second portion only after forty-eight hours, with positive results in every case. The thyroid colloid of the intoxicated animal gives the same iodin reaction seen in the test animals which had received intravenously serum from the hyperthyroid animal. This demonstrates the presence of iodin in the blood of the hyperthyroid animals,[7] and since the colloid takes iodin from the blood, the latter may be considered hyperiodized with respect to the colloid itself. The thyroid-fed animals were

6. The material used was very kindly supplied to us by Parke, Davis & Co., for which we wish to express our appreciation.

7. In this paper we shall use the term "hyperthyroid" reservedly to mean that condition produced directly or indirectly by the feeding of thyroid products.

undoubtedly hyperthyroid, as shown by the toxic symptoms. From these results we believe that the same qualitative conditions of thyroid excess may well exist in both animals, differing mainly in degree. While the thyroid-fed animals were seen to be definitely intoxicated, the animals receiving hyperthyroid serum showed no apparent change other than the difference in staining reaction of the colloid. Pure inorganic iodid medication does cause a positive thyroid colloid reaction as shown by Jones, but relatively enormous doses were given (1.5 to 4 gm. iodin as potassium iodid). In our feeding experiments the iodin administered as thyroid iodin was approximately 4 mg. The commercial thyroid extract used was guaranteed to contain not less than 0.2 per cent. iodin. We did not check this determination, but did find that there was no inorganic iodin present.

It was thought advisable to parallel the thyroid feeding experiments with a series in which there was given an amount of inorganic iodin equal to the amount of organic iodin administered in the form of thyroid extract. The results, as here tabulated, show that so small an amount of inorganic iodin gives practically no change:

Number	Treatment	Result
JT 47	7 mg. sodium iodid in six days	No change
JT 48	4 mg. sodium iodid in six days	Slight increase
JT 49	No treatment	Slight increase
JT 50	4 mg. sodium iodid in six days	Decrease
JT 51	4 mg. sodium iodid in six days	Slight increase

Jones administered from 2 to 5 gm. potassium iodid and still found some yellow colloid. In the thyroid-fed animals all of the colloid gave a deep blue reaction. This shows that iodin accumulates in the colloid much more readily when in a thyroid combination than as inorganic iodid. Whether the accumulated iodin comes from the administered material directly, or from other tissues under the stimulus of this administered material, we have not determined. Certain it is, however, that both commercial thyroids and inorganic iodids produce a similar reaction in the thyroid when administered to the animal *per os*. From a study of the Jones data on an iodin basis, the commercial thyroid product is seen to be more active with respect to production of a blue staining colloid than one thousand times as much iodin when given as potassium iodid. On the other hand, Jones could find no blue staining colloid in certain glands which, on analysis by the Baumann-Oswald method, gave some, though small, amounts of iodin. The advantage of this method obviously is that it is a microchemical test and is applicable to minute sections of thyroid, the loss of which would produce only a negligible effect on the unremoved portion. It has the disadvantage of necessarily assuming the thyroid colloid to be alike throughout the various areas of the whole gland. This, together with the fact that there is, in all prob-

ability, a small amount of iodin in the cells of the thyroid, probably explains the discrepancies in Jones' data compared with the actual iodin content. The results in our present series are so constant as essentially to eliminate this factor.

Since there is no known method of standardization of desiccated thyroid preparations on any basis other than that of their iodin content, it is not known definitely whether hyperthyroidism can be produced equally well with an iodin free thyroid product. The balance of opinion, however, is in favor of the necessity of the iodized component.[8] Certain it is that alkali iodids administered in equivalent dosages produce none of the signs of intoxication seen in experimental hyperthyroidism.

Serum from thyroidectomized rabbits when injected into normal test animals should produce in the blood of the latter a relative deficiency of thyroid products, and following physical laws should cause an outflow from the gland colloid. On the other hand, such serum might contain a physiological stimulus in the form of certain metabolic products which act specifically on the thyroid gland. Whatever the mechanism, we should expect either a decrease in the iodin of the gland or no change, depending on the individual ability to recuperate from the increased demands for thyroid secretion. This is just what was found. In four instances there appeared no demonstrable change, but in six of the animals there was observed a marked decrease in the iodin stain.

A mixed serum from several normal rabbits might be expected to produce in a series of normal animals any of the three possible changes: increase, decrease, or no change of the iodin content, dependent on whether the thyroid activity of the individual test animal was above, equal to, or below the common level of activity determined by the donors. This was the result obtained.

The accompanying tables need but little explanation. All rabbits marked with a common letter have received similar treatment and belong to the same series. A mixed serum from several animals was used in order to reduce to a common level all individual variations.

It is seen that of ten rabbits receiving serum from hyperthyroid rabbits, all show an increase of iodin except one, which was tested twenty-four hours after injection. This exception indicates that twenty-four hours is rather too short a time for demonstrable iodin changes to take place, though several gave positive tests within that time. At forty-eight hours there were no exceptions. How long the excess may be retained we have made no attempt to determine.

None of the rabbits receiving hypothyroid rabbit serum showed an increase in iodin, though in four instances there was no change, while the greater number showed an actual decrease.

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8. Stoland, O. O.: Am. Jour. Physiol., 1912, xxx, 37.

Of fifteen animals receiving normal rabbit serum, four responded with an increase in colloid iodin; the rest have shown either no change or a decrease.

All rabbits receiving the commercial desiccated thyroid *per os* showed increased iodin.

The rôle of the iodin in the thyroid gland is no more understood than that of the thyroid itself. The iodin may play a rôle in the thyroid secretion roughly comparable to the iron of hemoglobin. Hemoglobin is a non-toxic compound. Hematin, on the other hand, is quite toxic.[9]

TABLE SHOWING IODIN RESPONSE IN RABBITS RECEIVING VARIOUS SERUMS

Rabbits Receiving 5 c.c. Normal Rabbit Serum.				Rabbits Receiving 5 c.c. Hyperthyroid Rabbit Serum.				Rabbits Receiving 5 c.c. Thyroidectomized Rabbit Serum.			
No.	Wt. Gms.	Time, Hours.	Iodin Response	No.	Wt. Gms.	Time, Hours.	Iodin Response	No.	Wt. Gms.	Time, Hours.	Iodin Response
11.a	650-900	48	Increase	3.d	835	24	Increase	17.h	650-900	48	Decrease
13.a	810	48	Decrease	5.e	1700	24	Increase	18.h	650-900	48	No ch'ge
14.a	810	48	Increase	6.e	1800	24	Increase	19.h	650-900	48	No ch'ge
22.b	810	48	Decrease	7.f	1056	24	Decrease	20.h	650-900	48	Decrease
26.b	810	48	Decrease	8.f	1015	48	Increase	21.h	650-900	48	Decrease
27.b	810	48	No ch'ge	9.f	942	48	Increase	23.h	650-900	48	Decrease
28.b	810	48	Decrease	10.f	942	48	Increase	38.i	768	48	No ch'ge
34.c	818	48	Increase	29.g	1234	48	Increase	39.i	510	48	Decrease
35.c	720	48	Decrease	30.g	1000	48	Increase	45.i	985	48	Decrease
37.c	572	48	Increase	31.g	975	48	Increase	46.i	850	48	No ch'ge
36.c	820	48	Decrease	32.g	679	48	Increase				
40.c	795	48	Decrease	33.g	700	48	Increase				
41.c	907	48	Decrease								
42.c	910	48	Decrease								
43.c	915	48	Decrease								
44.c	880	48	Decrease								

SUMMARY		SUMMARY		SUMMARY	
Total inc. iodin	4	Total inc. iodin	11	Total inc. iodin	0
Total no change	1	Total no change	0	Total no change	4
Total dec. iodin	11	Total dec. iodin	1	Total dec. iodin	6

The iodin may be so combined in the normal secretion of the gland as to form a physiological product, non-toxic in character, while in abnormal conditions, as in diseased thyroids or in the toxic desiccated glands, it may be split off into a smaller molecular grouping which may itself be toxic, or may be accompanied by a non-iodized substance which is responsible for the toxic effects. The list of possibilities is unlimited.

9. Brown, W. H.: Jour. Exper. Med., 1912, xv, No. 6.

To carry the analogy a step farther, the therapeutic value of official preparations is as a hematopoietic stimulant; correspondingly it is observed that in certain thyroid diseases iodin possesses a marked therapeutic value. As iron is for the most part found in hemoglobin, so is the greater part of the iodin found in the thyroid, doubtless as iodothyrin or some closely related form. It is probable, then, that the increase of iodin in the colloid following artificial hyperthyroidism means an increase in organic iodin compounds of the thyroid.

Jones has shown that manipulative procedures, such as operations, and particularly shock, are conducive of a decrease of iodin. Yet this is an almost negligible factor, owing to the relatively slight trauma associated with a partial thyroid removal. The animals are usually sufficiently recovered within one-half hour to eat their food unconcernedly. The fact that our manipulative procedures effect no change in the colloid iodin has been controlled histologically.

Another factor which may have operated to cause a predominance in iodin loss in the normal series, is the fact that we have employed only adult rabbits for our source of normal and hypothyroid rabbit serum. Both adults and young were used in the production of a hyperthyroid serum and with the same result in both cases. If, as is generally supposed, the adult thyroid is normally less active than the young thyroid, then adult normal serum might be expected to be hypothyroid with respect to the plane of activity of the young rabbits employed as test objects. It would seem advisable in this connection to control by means of injections of serum of young rabbits into adult test animals.

If exophthalmic goiter is to be considered hyperthyroidism with an excess of an iodized thyroid secretion, we ought to be able to show it by an appropriate modification of our methods. Work along this line is now in progress.

SUMMARY

1. The relative iodin content of the thyroid colloid is determined by Mallory's connective tissue stain as applied by Jones.

2. The iodin content of the thyroid glands of rabbits serving as test animals was found to be increased directly by feeding commercial desiccated thyroids, and indirectly, by intravenous injections of serum from hyperthyroid rabbits.

3. The iodin content of the thyroid gland was found to be decreased by intravenous injections of serum of thyroidectomized rabbits.

4. Following normal serum injections, the iodin content was sometimes increased, sometimes decreased, or remained constant, indicating individual variations in thyroid activity.

The authors wish to express their appreciation of the kindly interest and criticism of Professors A. S. Loevenhart and C. H. Bunting.

THE SOURCE OF URINARY INDOL-ACETIC ACID IN TWO DEMENTIA PRAECOX PATIENTS*

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In this laboratory indol-acetic acid has been found in 21 per cent. of ninety-one urines of healthy normal persons.[1] Of 174 urines from dementia praecox patients 48 per cent. contained indol-acetic acid. The occurrence of the compound in the urine seemed to be related to a peculiar constitutional condition.

Hopkins and Cole[2] showed that *B. coli*, under partial aerobic conditions, would produce indol and indol-acetic acid from tryptophan. Salkowski[3] showed that if indol-acetic acid were given by the mouth, it immediately appeared in the urine. Herter[4] reported indol-acetic acid in the urine of an under-developed child suffering from an excessive and peculiar form of intestinal putrefaction. Putting these facts together, the natural conclusion would be that indol-acetic acid in the urine is a result of its production in the intestine by intestinal putrefaction of foods containing tryptophan. If this conclusion were true, indol-acetic acid, like indican, should be present in all urines, the quantity being dependent on the amount of intestinal putrefaction, and should be found in large quantities almost as frequently among sane as insane persons. In testing nearly 600 urines for indol-acetic acid over 50 per cent. gave no reaction for the compound.[1] A number of qualitative tests for indol-acetic acid and indican have not shown a high indican content to be always accompanied by a high indol-acetic acid content. So the following questions present themselves: (1) Is indol-acetic acid derived directly from ingested protein, and (2) is it derived from intestinal putrefaction, such as produces indican? In other words, is indol-acetic acid endogenous or exogenous?

EXPERIMENTAL

Urorosein produced from indol-acetic acid in the urine by the action of hydrochloric acid and a small amount of an oxidizing agent, has a

* Preliminary Article from the Laboratory of the Illinois State Psychopathic Institute.

* Submitted for publication June 23, 1913.

1. Ross: Preliminary Note on Excretion of Indol-Acetic Acid in the Urine. THE ARCHIVES INT. MED., 1913, xii.
2. Hopkins and Cole: Jour. Physiol., 1903, xxix, 451.
3. Salkowski: Ztschr. f. Physiol. Chem., ix, 26.
4. Herter: Jour. Biol. Chem., 1908, iv, 239.

bright red color. It was suggested to me by the director of the laboratory, Dr. H. Douglas Singer, that the intensity of the reaction might be measured with the Fleischl-Miescher hemoglobinometer. It was found that this could be done with a degree of exactness which compared very well with ordinary quantitative chemical analyses.

The next problem to solve was to evaluate the units of the scale of the hemoglobinometer in terms of indol-acetic acid. This was accomplished by extracting a large quantity of urorosein from the urine with amyl alcohol. The amyl alcohol solution was treated with solid sodium hydroxid till the red color disappeared. The solution was filtered, and carbon dioxid passed through the filtrate till no more precipitate was formed. This solution was filtered and evaporated to dryness at room temperature. The residue was thoroughly washed repeatedly with ether, chloroform and xylene. The residue was then dissolved in ethyl alcohol, filtered and made up to a volume of 100 c.c. in a measuring flask. Measured volumes of the solution were run through the Kjeldahl method for nitrogen. Measured volumes were evaporated to dryness and the residue dissolved in measured volumes of amyl alcohol which had been saturated with HCl containing sufficient sodium nitrite to bring a maximum color. This red solution was then put into the 12 mm. chamber of the hemoglobinometer and the readings taken, using electric light as the luminant. A number of these determinations were done and the averages calculated. These averages and the empirical formula of indol-acetic acid, gave sufficient data to calculate the value of a single unit on the scale of the Fleischl-Miescher hemoglobinometer in terms of indol-acetic acid. The result was that each hemoglobinometer scale unit indicated .0788 milligrams of indol-acetic acid corresponding to the urorosein in 1 c.c. of the amyl alcohol solution.

Empirical Method of Determining Indol-Acetic Acid: Twenty-five c.c. of urine were clarified with 5 c.c. of basic lead acetate[5] and filtered. Twenty c.c. of concentrated hydrochloric acid and 5 drops of 0.2 per cent. sodium nitrite were added and shaken for five minutes. The amyl alcohol was let out into a filter from which the hemoglobinometer chamber was filled. The Fleischl-Miescher hemoglobinometer with the 12 mm. chamber was used. An electric light was employed as the luminant. Using the scale unit value of the hemoglobinometer as determined above, the total indol-acetic acid in the urines collected each day was calculated. The whole process was carried out as rapidly as possible. Ten readings were made on each determination.

5. The basic lead acetate was made up by the official methods of the chemists of the U. S. Department of Agriculture and then diluted with 2½ volumes of water.

I do not claim this method to determine absolutely all of the indol-acetic acid of the urine, nor the standardization of the scale units of the hemoglobinometer to be perfect. More work to this end is being done in this laboratory. It is, however, claimed that the method is accurate in determining the relative amounts in different urines. These points should be kept in mind in the consideration of the values given in the accompanying tables:

TABLE 1.—INDOL-ACETIC ACID AND INDICAN EXCRETION PER DAY

Date*	Patient M. P.		Patient M. O'B.	
	Indol-Acetic Acid in Milligrams	Indican in Milligrams	Indol-Acetic Acid in Milligrams	Indican in Milligrams
May 15	.....	.....	.....	.....
16	79.51	†	53.78	†
17	78.45	.....	46.39	.....
18	97.97	.....	64.75	.....
19	161.11	16.88	97.36	11.90
20	171.97	56.28	79.61	53.52
21	121.52	47.36	86.97	35.51
22	124.49	60.34	85.48	55.71
23	.....	.....	.....	.....
24	81.33	35.66	99.73§	17.76§
25	111.97	30.07	76.53	18.85
26	167.64	28.27	123.30	15.38
27	198.25	8.41	119.47‡	4.33‡
28	98.39‡	25.64‡	65.73	9.25
29	97.46	38.70	57.73	19.18
30	72.45	56.38	79.31	29.39
31	102.40	46.44	98.48	26.75

* Horizontal lines divide the periods. May 15 and 23 the losses were too great for determinations to be made.

† Not accurately determined.

‡ Indicates the loss of one or more urinations; in this instance the loss of one urine out of nine. These values are computed by multiplying the results on the urine collected by the fraction: $\frac{\text{Total urinations}}{\text{Total urinations collected}}$

§ Loss of two urines out of six.

The method of determining the amount of indican excreted was that of Ellinger as described by Dr. P. B. Hawk.[6]

6. Hawk, P. B.: Text-Book of Practical Physiological Chemistry, Edition 3, p. 387.

Determinations of indol-acetic and indican were made on each twenty-four-hour specimen as soon as collected. Duplicate determinations were made in each case.

The subjects of this test were two women patients in the Kankakee State Hospital. Both were classed in the dementia praecox group. Both patients at the time of the test seemed to be in perfect physical condition. Their body weights were nearly the same, each being between 100 and 110 pounds. During the test the patients were given a walk of nearly two miles each day.

TABLE 2.—INDOL-ACETIC ACID AND INDICAN EXCRETION PER DAY

Period	Patient M. P.		Patient M. O'B.	
	Indol-Acetic Acid in Milligrams	Indican in Milligrams	Indol-Acetic Acid in Milligrams	Indican in Milligrams
Tryptophan-free diet. Period I	104.26	Lost	65.57	Lost
High protein diet. Period II	139.33	54.66	84.02	48.25
Near protein-free diet. Period III ..	139.79	25.58	104.76	14.08
High protein diet. Period IV	92.67	41.79	75.31	21.14

The plan of the work was to determine the amounts of indol-acetic acid and indican excreted in the urine in twenty-four hours, while being nourished, first on tryptophan-free diet, second on a high protein diet, third on a non-protein diet, and fourth on high protein diet supplemented with agar to insure frequent bowel movements. The diet of the first period of four days consisted of corn-starch, tapioca, butterine, sugar, beef extract, gelatin, tea and coffee. The food of the second period of four days consisted of eggs, beefsteak, cheese in large amount, ham, oatmeal, a little potato, toast, coffee and tea. The food of the third period of four days was made up of corn-starch, tapioca, sugar, butterine, salt, very little beef extract, coffee and tea. The diet of the fourth period of five days consisted of eggs, beefsteak, oatmeal, potatoes (small amount), toast, coffee and tea. Roughly calculating the protein intake during the second and fourth periods it was at least 120 gm. per day, and for the third period not over 5 gm. per day per person.

The results of the analyses for indol-acetic acid and indican for the test are shown in Table 1.

It will be noted from the preceding table that it took one day after each change of diet for the indican output to be modified by the difference in diet. It is thought, therefore, that the results of the different diets will be most truthfully shown in the average of the daily output of the periods, including the first day after each change of diet in the preceding period. The results are as shown in Table 2.

The indican output was proportional to the protein ingestion, as would be expected. On the contrary, the indol-acetic acid output apparently did not vary directly with anything. It will be noted that the percentage of variation in the indol-acetic acid excretion was very small considering the extreme variations in diet. In view of this, it is suggestive that urinary indol-acetic acid excretion is more or less constant for the individual in a similar way to that of creatinin.

CONCLUSION

Urinary indol-acetic acid in the case of these two patients was endogenous.

CULTURAL RESULTS IN HODGKIN'S DISEASE*

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The publication at this time of the result of our endeavors to cultivate an organism from the tissues of patients with Hodgkin's disease is occasioned by the publication by Negri and Mieremet[1] of the successful cultivation from two cases of Hodgkin's disease of a diphtheroid organism which they designate as the *Corynebacterium granulomatis maligni.*

While Hodgkin's disease, or malignant granuloma of the lymphatic apparatus, has of late years come to be generally regarded as of infectious nature, the etiological agent has remained undetermined. The work in this country of Reed[2] and of Longcope[3] showed definitely that the condition was independent of tuberculosis, and that the theory supported by Sternberg[4] that it was a manifestation of the activity of the tubercle bacillus could not be maintained.

In 1900 Fraenkel and Much[5] reported that by treating Hodgkin's nodes with a strong alkalin solution of sodium hypochlorid (antiformin) they had found in the sediment, in twelve out of thirteen cases, certain granular, Gram-staining, but non-acid-fast bacilli, which they considered to be non-acid-fast tubercle bacilli, "possibly identical with the ordinary tubercle bacillus," but "more probably a special form of the tubercle virus," or at least "belonging to a related group of organisms."

In the present year Negri and Mieremet[1] have reported the successful cultivation on Bordet's medium (blood-glycerin-potato-agar), from two cases of Hodgkin's disease, of an organism which falls within the diphtheria group and further agrees in morphology with the forms described by Fraenkel and Much. The organism is a non-acid-fast, Gram-staining bacillus, growing luxuriantly at body temperature, and is a facultative anaerobe. Perhaps its most striking feature is its pleomorphism. The organism showed such variability in form that it was only after isolation of a single organism and cultivation of it that the authors were satisfied that they were dealing with a pure culture. In various media the follow-

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* From the Pathological Laboratory of the University of Wisconsin.
* Paper read before the Association of American Physicians, May 7, 1913.
* This work has been aided by a grant from the Rockefeller Institute for Medical Research.

1. Negri and Mieremet: Centralbl. f. Bakteriol., 1913, lxviii, 292.
2. Reed: Johns Hopkins Hosp. Reps., x, 133.
3. Longcope: Bull. Ayer Clin. Lab., 1903, No. 1.
4. Sternberg: Ztschr. f. Heilk., 1898, xix, 21.
5. Fraenkel and Much: Ztschr. f. Hyg., 1910, lxvii, 159.

ing forms were found: Plump, short rods, some so short as to resemble cocco-bacilli, these latter forms predominating or being exclusively found in old cultures; small thin bacilli with polar-staining; comma-shaped bacilli; granular rods of variable size, often of considerable length; branching forms; and on all media, club-shaped involution forms and large spherical forms. This organism is resistant to antiformin and the authors consider it identical with the organism described by Fraenkel and Much.

In a study of Hodgkin's disease, extending over the past five years, we have made various attempts to secure cultures from material removed at autopsy and at operation. The first attempts were made with ordinary media, and were unsuccessful. In Case 9 of our reported series,[6] cultures were made at operation, in October, 1910, by planting portions of lymph-nodes in bouillon, but apparently in this case there was a complicating infection, as only a streptococcus growth was obtained. In Case 5 of the series, attempts were made to secure cultures from the enlarged mesenteric nodes at autopsy, in January, 1912, but post mortem invading organisms completely overgrew the media.

After these failures, it was felt that special media must be used, and as at this time we felt from certain observations that the organism was probably one of the higher forms of bacteria, if not a fungus, media appropriate to the growth of these organisms was selected. Dorset's egg-medium and glycerin-phosphate-agar were first tried. The first opportunity for their use came in an operation by one of us (J. L. Yates) for the removal, on Feb. 13, 1912, from a woman patient, of a large group of cervical lymph-nodes.

The essential points in her history are as follows:

CASE 1.—Female, white, age 33. When first seen in October, 1911, the pátient showed in the right side of her neck a group of enlarged, discrete, lymph-nodes extending from the mid-clavicular region upward in the posterior triangle practically to the attachement of the sternocleido-mastoid. This group of nodes had shown progressive enlargement during the year previous. Except for one small node below the clavicle on the right side and a rather large gland in the right axilla, no further enlargement of lymph-nodes was noted at this time. The spleen was not palpable. The tonsils were enlarged. October 10, a von Pirquet test and a Wassermann reaction were both negative. October 23 the large axillary node was removed for diagnosis. In section it showed hyperplasia of lymphoid and of endothelial elements with giant cells of the endothelial type and of the megalokaryocyte type, disappearance of the architecture of the node, and a beginning diffuse sclerosis and marked eosinophilic infiltration. December 11 tonsillectomy was done, and Feb. 13, 1912, the large group of lymph-nodes in the right side of the neck with the intervening sclerotic tissue, was removed en masse.

After removal the group of nodes was incised, and, with the observation of the strictest aseptic technic, portions of the nodes and of the interglandular tissue were planted on the surface of slants of the egg-medium and of the glycerin-

6. Bull. Johns Hopkins Hosp., 1911, xxii, 368.

phosphate-agar. The tubes were sealed with paraffin and incubated at 37 C. The tubes were apparently sterile at the end of ten days' incubation, but they were left undisturbed in the incubator, and at the end of three weeks' time a slight growth was noted on one tube. March 12, two of the egg tubes and one tube of glycerin-phosphate-agar showed pure cultures of a diphtheroid organism, with many clubbed-shaped involution forms, with granular and barred rods and evidently branching filaments. The organism was Gram-staining.

Subcultures were made from the original tubes on blood-serum, egg, glycerin, agar, and the ordinary media. The organism proved to be a rather feeble grower under the conditions under which its growth was attempted at that time. On blood serum in twenty-four hours there were obtained the characteristic shining, opaque, white colonies. On agar the growth was slow, the streak at first being of a grayish, glistening, translucent appearance, becoming more opaque with age. In bouillon there was a scanty growth which gradually settled to the bottom as a somewhat slimy precipitate. No turbidity of the medium occurred. Milk was not coagulated. Its reaction was changed but little if any.

The growth was tested on guinea-pigs, and while the results were unsatisfactory in a sense, it was determined that the animals would survive inoculation with a heavy dosage of a twenty-four-hour culture for ten days at least. Through an accident this culture was lost before thorough testing of it could be done.

Further opportunities for obtaining cultures were not obtained until January, 1913, when, through the kindness of Dr. W. J. Mayo and the cooperation of his staff, the courtesies of the clinic at Rochester were extended to us.

On Jan. 9, 1913, cultures were made from a cervical gland removed for diagnostic purposes from a patient with the following history:

CASE 1.—Female, white, aged 34 years. Four years previously she noticed a swelling on the right side of her neck, followed by the appearance of other small tumors. Ten months before date enlarged lymph-nodes appeared on the left side of the neck and in the right axilla. Six weeks previously nodes appeared in the left axilla. Physical examination revealed, in addition to these nodules, a mediastinal mass, a six months' pregnancy, over which were felt discrete firm nodules which slipped under the examining hand. Nose and throat examination revealed a hypertrophic rhinitis, causing some nasal obstruction and also enlarged tonsils. Red blood-cells, 4,480,000; leukocytes, 12,400.

Pathology of Node.—The test node shows microscopically loss of architecture, with lymphoid and endothelial hyperplasia, the presence of many endothelial giant cells, a beginning fine sclerosis and eosinophilic infiltration. There is slight invasion of the capsule.

Pieces of the gland were planted on egg-medium and on Loeffler's blood-serum. The tubes were sealed and incubated. On January 17 one egg-tube showed the characteristic clubbed and granular diphtheroid forms seen in the first culture; but associated with a growth of *B. subtilis*. One serum-tube showed a pure culture of the diphtheroid organism, and one serum tube showed the presence of a white staphylococcus. Three other tubes were sterile.

Subcultures of the diphtheroid organism were made from the serum tube on to other media, particularly egg-medium, glycerin-phosphate-agar and plain agar. The growth proved to be luxuriant on these. The top of the streaks had a grayish glistening appearance, while the lower part of the streaks were at first of an opaque white appearance, gradually assuming a slight yellowish color, deepening in very old cultures, as glycerin-agar, to a brownish. Smears from the older cultures showed such a pleomorphism, and there were so many coccoid forms it was at first thought we were dealing with a mixed culture of bacillus and coccus. It was only after growth of many generations on egg-medium, on which the culture is of a pure bacillary type during the first twenty-four hours, and after shaking the culture with sand and plating from a

further dilution, which gave colonies of a uniform and characteristic type, that we became assured we were dealing not with a symbiosis of coccus and bacillus, but with a pleomorphic organism.

A third successful cultivation was made Feb. 15, 1913, from a cervical lymph-node from the following case from the Mayo Clinic:

CASE 3.—Married female, white, aged 38, with marked enlargement of the cervical nodes and rather general glandular enlargement. The illness began with involvement of the cervical glands two years previous to date.

The node removed for diagnosis showed extreme sclerosis, but with scattered alveoli-like areas in which the characteristic elements of the Hodgkin's lymph-node, the endothelial cells, giant cells and lymphocytes were preserved. Eosinophilic infiltration was well marked.

After one week's cultivation there were secured from two tubes, one of Loeffler's serum and the other of egg-medium, a pure culture of a diphtheroid, pleomorphic organism apparently identical with that secured from the previous case, showing the same cultural characteristics. It was put through the same procedure to determine the purity of the culture and gave the same result, that is, after shaking with sand and diluting and plating, all the colonies on the plates were of a uniform character.

In the interval between the last two cases, attempts were made to secure cultures from two other cases, but they resulted less successfully. The first of these attempts was at a post-mortem, Jan. 15, 1913, on the following case:

CASE 4.—Female, white, aged 50 years (patient of Dr. Reeves of Albany, Wis.). The glandular swelling had first appeared in the left cervical region two years before. The cervical group of lymph-nodes had been removed about six months previous to the patient's death. At the post-mortem examination there was found rather general glandular involvement, but especial enlargement of the mesenteric and retroperitoneal nodes and of the lymphoid elements of the spleen. There was also a chylous ascites, well marked anemia and emaciation. Old pleural scars were noted at the pulmonary apices and one calcified focus in a bronchial lymph-node. No other signs of tuberculosis were seen.

Microscopically, the abdominal lymph-nodes showed a well-marked hyperplastic Hodgkin's disease picture with beginning diffuse sclerosis, while the splenic lesions showed a more advanced sclerotic condition.

On one Loeffler serum-tube, in which was planted a piece removed from the interior of the spleen, there was noted a growth on the tissue itself of typical polar-staining diphtheroid organisms, but for some reason unascertained, they would not "catch on" to the medium, and attempted subcultures from scrapings of the tissue on both the egg-medium and on serum proved negative.

The second case was the following from the Mayo Clinic, Jan. 31, 1913:

CASE 5.—Male, white, aged 33. The patient had had an abscess of a tooth six months previously. About three weeks before coming to the hospital patient had noticed a painless swollen node in the left supraclavicular region. This had increased in size during the time of observation. The large node removed was of a uniform medullary appearance on gross section, and microscopically showed lymphoid and endothelial hyperplasia, with the presence of giant cells, beginning diffuse sclerosis and eosinophilic infiltration. The architecture of the node was destroyed.

Unfortunately for the success of cultural investigation, this gland had been handled and incised before the plants on the media were made. Four days after

incubation of the tubes was begun, there was found on one of the serum-tubes a growth of the typical diphtheroid organism, but in association with a coarse bacillus of the *B. subtilis* type. As at this time our efforts were being directed to the elimination of the supposed coccoid contamination of our second culture, no attempt was made to separate the diphtheroid organism from this last mixed culture.

While we have made no systematic attempt to find the organism described in histological preparations from the nodes of Hodgkin's disease patients, one case should be recorded as of interest in connection with the cultural results. Portions of the organs from a girl of 6 years, a patient of Dr. Ogden of Milwaukee, who died in a severe vomiting attack, were received in the laboratory. The Peyer's patches of the intestine and the mesenteric lymph-nodes showed the changes of early Hodgkin's disease, which from clinical and post mortem evidence must have been primarily intestinal in origin. On staining the sections by the Gram-Weigert method, the only organisms found, save for the surface intestinal bacteria, were groups of polar- and granular-staining diphtheroid organisms lying deep within the enlarged Peyer's patches.

SUMMARY OF CULTURES

To summarize our cultural results: In three cases of Hodgkin's disease we have secured a pure culture of a pleomorphic diphtheroid organism. In two other cultural attempts the organism was recognized, but was not secured in pure culture, and in a sixth case a similar organism, morphologically, was stained in the lesions of a primary intestinal Hogkin's case.

The detailed biological reactions of this diphtheroid organism have as yet not been completely worked out by us. The strain recovered from the second case, however, has been found to grow readily at 37 C. on the media used to secure the cultures and on ordinary agar-agar. On glycerin-phosphate-agar the growth is almost as luxuriant under strict anaerobic as under aerobic conditions. For luxuriant growth, marked moisture of the medium seems necessary. On a relatively dry medium, growth is slow, and the organisms are found to develop as the long forms, granular, banded, and with many club-shaped involution forms. Branching forms are also noted. These forms are especially well developed on the egg-medium, where they also seem to have a tendency to cohere, so that in stained smears one gets many small groups of organisms radially arranged, with clubbed peripheral elements, somewhat suggestive of a minute actinomyces colony, as seen in section. On moist serum tubes with luxuriant growth, the organisms are short and plump, with polar staining. Many of these forms are coccoid. We have noted also,

as emphasized by Negri and Mieremet, that in all old cultures coccoid forms predominate, and also that large spherical involution forms are present. A colony or a streak, which at twenty-four hours shows entirely the bacillary form, will twenty-four or forty-eight hours later show an apparent outnumbering of the bacilli by the coccoid elements.

The organism stains by the Gram method, though the short plump forms hold the dye less strongly than the longer bacillary forms. It is not acid fast. No spore formation has been noted.

The growth of the organism is at first glistening and grayish, but becomes more opaque and of a white color. Apparently, depending somewhat on the reaction of the media, there may be in some early cultures a slight greenish-yellow tint produced. Old cultures on glycerin-phosphate-agar become brownish, and the media itself darkens.

Gelatin is not liquefied. There is no early change in reaction in litmus milk. Bouillon is not clouded by the growth. Flecks appear along the side of the tube, and a slimy deposit gradually accumulates at the bottom.

Plate cultures show a rounded colony with quite regular edge, a fine stippling of the growth and a central dark spot. The colonies are of a glistening gray color at the end of twenty-four hours, becoming gradually of an opaque white color.

Altogether, our studies thus far seem to indicate that we are dealing with the same organism described by Negri and Mieremet, and while our results taken with theirs cannot be said to indicate positively that we are dealing with the cause of Hodgkin's disease, yet they are strongly suggestive. The morphological elements obtained by Fraenkel and Much by the antiformin method in twelve out of thirteen cases are so similar to the organism cultivated by us that we find added support in their findings to the importance of the organism in the disease. Ultimate proof can come only from an extended series of cultures, or from animal experimentation. The latter test is now in progress in this laboratory with a variety of animal species. Should the relation of the organism to the disease be established, we would suggest *Corynebacterium Hodgkini* as a more appropriate name for the species than the trinomial designation suggested by Negri and Mieremet.

Although the utmost efforts were made to prevent carrying in organisms from the skin, when removing nodes for cultural investigation, in almost every case studied, one or more tubes have shown the presence of a white staphylococcus. This, when taken in connection with the polymorphonuclear leukocytosis, which occurs late in the disease, has suggested that possibly a secondary infection plays a part in the development of the disease. This feature also needs further investigation.

ADDENDUM

After this paper was placed in the hands of the publishers, the opportunity presented to make cultures in another case of Hodgkin's disease from a patient in the charge of Dr. Frank Billings of Chicago. The essential points in the patient's history are as follows:

Male, white, aged 32. Loss of weight since Jan. 1, 1913. During a period of six weeks before date of examination (April 23, 1913) gradual enlargement of the nodes in the neck and groin and to some extent in the axillae was noted. On examination there was found moderate enlargement of the tonsils, cervical, axillary and inguinal nodes and a firm, palpable spleen.

April 28, blood-count was, R. B. C., 4,150,000; W. B. C., 4,150. Hgb., 70 per cent. (Dare). April 28 a node from the left side of the neck was removed by Dr. Dean Lewis. This showed on section endothelial proliferation with endothelial giant cells, some fine diffuse sclerosis and but little eosinophilic infiltration.

May 7, Dr. F. B. Moorehead extracted right upper first molar and second bicuspid teeth, finding a large amount of granulation tissue around the roots, and along the lingual root of the molar an abscess cavity about 1 cm. in length containing pus of foul odor. Unfortunately, no cultures were made.

May 29, with the observation of the strictest precautions to prevent contamination, Dr. Lewis removed a node from the left cervical region, and one from the right inguinal region.

In the laboratory of Dr. E. C. Rosenow, and with his assistance, which is gratefully acknowledged, pieces of these nodes were planted on Loeffler's serum, egg-medium, blood-agar and serum-glucose-agar. Other portions of the nodes were ground in a sterile mortar and the emulsion planted on tubes of the media mentioned.

From these plants growth of the diphtheroid organism was obtained in pure culture on Loeffler's serum from both the cervical and the inguinal nodes where solid pieces of the node were used. The organism was also obtained from the cervical node, where the emulsion was planted, but with a contaminating pigment-producing air organism.

Dr. Rosenow reports positive results from the cervical node on blood-agar slants, aerobic and anaerobic, and from the depths of one tube of serum-glucose-agar. From the inguinal node two colonies developed in the depths of the serum-glucose-agar tube.

BOOK REVIEW

DIGESTION AND METABOLISM. The Physiological and Pathological Chemistry of Nutrition. For students and physicians. By Alonzo Englebert Taylor, M.D., Rush Professor of Physiological Chemistry, University of Pennsylvania, Philadelphia. Octavo, 560 pages. Cloth, $3.75 net. Lea & Febiger, Philadelphia and New York, 1912.

The medical profession of America owes Dr. Taylor a debt of gratitude for making available to those who read only English the vast extent of chemical knowledge of the processes of digestion and nutrition which he has put between the covers of his book. The general plan of the work, the sequence of the chapters, the completeness with which he has treated each subject, and the vigorous and positive style which Dr. Taylor always has at his command, all contribute to make it a noteworthy text-book. To the reviewer the systematic use of full chemical formulas throughout the book seems especially commendable. They conduce to visualization of the actual chemical processes described and, therefore, educate the reader to think chemically, a much needed ability for the modern physician.

With so much that is admirable to praise, it seems ungracious to ask for more; yet the reviewer cannot but regret profoundly that Dr. Taylor was content to produce only a text-book. That he did this advisedly, he says in his preface, and that after one-third of the work was completed as a book of reference. Only then did he determine to omit all statement of his authorities and his bibliography. Had he contined as he began, the book would have been of the utmost value to American medical science, which is becoming especially strong in the field of physiological chemistry, and would have supplemented in a welcome manner Lusk's Science of Nutrition. Only in America would a man of Professor Taylor's learning think of producing a work of this magnitude in the form of a student's text-book. It is far too exhaustive and detailed to meet the need only of learners, and it is to be hoped that Professor Taylor may be persuaded to add his references in a future edition, in order that the usefulness of his work may extend to his fellow-investigators as well. Even for the American student, separated as he is from contact with foreign investigators, it is most desirable that he should become familiar with their names and their contributions to the knowledge which is handed on to him for use.

In certain details the lack of citation of authority is particularly felt. On pp. 239-240, in a rather inadequate discussion of the formation of sugar from amino-acid, this is true. The statement that the human diabetic gets two or three times the amount of sugar from protein that the normal body does, cannot be accepted without a fuller discussion. On page 259 one finds this statement: "The ingested sugar must in these cases be burned, if resorbed, since there is no glucosuria and, therefore, no hyperglucemia. Typical instances of acute exophthalmic goiter seems to behave in the same way." As a matter of fact, the investigation of the blood sugar in exophthalmic goiter is revealing hyperglucemia with great frequency. The reviewer would have been much indebted to Professor Taylor for his references to these typical acute cases. On page 264 he states: "We know that when the pancreas is removed the muscles cannot burn glucose properly. This positive fact is far more fundamental than the negative results of test-tube experiments with extracts of muscle and pancreas." Unfortunately, there is at present much uncertainty about this fundamental "positive fact," which has recently been denied by von Noorden. Though few would agree with the latter's views, they do not make definite statement desirable, without full discussion and references to the sources of knowledge on the subject. Also, on

page 271, reference is made to the presence of acetone in the blood. This is usually denied.

However, the whole discussion of the combustion of glucose is written in a most interesting way, as is the chapter on ferments, Professor Taylor's special interest. The present state of knowledge in each field is well presented, and the information, as far as can be judged without a statement of sources, is accurate. No one, student or investigator, can read the book without real pleasure and great profit, and it is to be hoped that many will be induced to. Some errors demand correction in the next printing. On page 47, "arginin" is spelled "argenin." On page 248 the equation "glucose + water = glycogen" is reversed. On page 265 "intestinal secretion" should be "internal secretion." Professor Taylor also coins some words to which the reviewer objects, especially "input" for intake. But these are insignificant criticisms of a book which has been read with unusual interest.